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GLOMAL TUMORS

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Up to recent times the glomal tumor was considered quite rare. No doubt in the past many cases were overlooked or incorrectly classified. As a result of Masson's [1] revealing work in 1924, this peculiar combination of structures, so clearly described by him, has been accepted as a definite entity, and numerous instances of it have been described in the recent literature. The following tabulation will give an idea of the number reported to date.

	Reported	Collected
Adair [2]	12 (one with 3)	
Bailey [3]	7	1
Geschichter [4]	..	1
Burman and Gold [5]	2	
Grieg [6]	3	15
Horsley [7]	1	
Jirka and Scuderi [8]	1	
Love [9]	1	
Lewis and Geschichter [10]	19	
Raisman and Mayer [11]	3	10
Stout [12]	11	
Radasch (present article)	4	

The total number reported is ninety, among which four were instances of multiple tumors.

LOCATION

The glomal tumors have had a wide distribution, that is, from the head to the soles of the feet; they have been encountered in the nail-bed and palmar surfaces of the fingers, palmar fascia, forearm,

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Read before the Pathological Society of Philadelphia, Nov. 12, 1936.

From the Laboratories of the Daniel Baugh Institute of Anatomy of the Jefferson Medical College.

1. Masson, P.: Lyon chir. **21**:257, 1924.
2. Adair, F. E.: Am. J. Surg. **25**:1, 1934.
3. Bailey, O. T.: Am. J. Path. **11**:915, 1935.
4. Geschichter, C. F.: Internat. Clin. **2**:1, 1936.
5. Burman, M. S., and Gold, A. M.: New York State J. Med. **5**:18, 1935.
6. Grieg, D. M.: Edinburgh M. J. **35**:565, 1928.
7. Horsley, G. W.: South. Med. & Surg. **98**:5, 1936.
8. Jirka, F. J., and Scuderi, C. S.: J. A. M. A. **107**:201, 1936.
9. Love, J. G.: Proc. Staff Meet., Mayo Clin. **10**:393, 1935.
10. Lewis, D., and Geschichter, C. F.: J. A. M. A. **105**:775, 1935.
11. Raisman, V., and Mayer, Leo: Arch. Surg. **30**:911, 1935.
12. Stout, A. P.: Am. J. Cancer **24**:225, 1935.

elbow, upper part of the arm, thigh, knee, lower part of the leg, toe and sole. They have occurred predominantly, however, in the upper extremities, especially in the fingers. They seem to occur mainly in adults.

APPEARANCE

When not deeply placed, glomal tumors appear as rounded or oval small masses, rather smooth in contour and of a rose or purple color; they are usually freely movable and solid, although at times they resemble hemangiomas. Each seems to be entirely encapsulated against the surrounding tissues. When subungual, they are smaller, measuring from 2 to 6 mm. in diameter; when not under such restraint, they are from 3 to 12 mm. long and from 1 to 5 mm. thick. Recently Lewis and Geschichter [10] described one that was the size of a lima bean.

SYMPTOMS

These neoplasms are from exceedingly to excruciatingly painful. The pain may be more or less constant, or it may be intermittent. It is usually not local but extensive; it may extend from the finger to the arm and shoulder and even into the head. These structures are also very painful to the slightest touch, especially of the clothing. There may also be an increase of temperature on the affected side. There is usually a history of trauma.

CLASSIFICATION AND ANATOMY

Glomal tumors were classified as angioma, painful subcutaneous tubercle and angiosarcoma until Masson called particular attention to them. Although his work was received with little enthusiasm at the time, it has now borne fruit, and his classification has been accepted. Of late, considerable interest has been aroused; a number of cases have been reported, and much knowledge in regard to the neoplasms has been added to the literature.

The glomal tumor represents hyperplasia of a normal structure, the arteriovenous anastomosis (digital glomus), and its structure resembles that of a gigantic glomus coccygeum. Anatomically, arteriovenous anastomoses are divided into two groups:

1. To this group belong the peripheral anastomoses described by Sucquet [13] and Hoyer.[14] The peripheral anastomosis is a normal structure that is characterized by a coiled and twisted appearance of the main vessel (the canal) and by a peculiarity of the structure of this channel, which differs widely from the ordinary structure of an artery, vein or capillaries. The diameter never exceeds 0.1 mm. In man such

13. Sucquet, J. P.: Bull. Acad. roy. de med. de Belgique **26**:825, 1860.
14. Hoyer, H.: Denkschr. d. warschauer M. Gesellsch. Jahresb., 1873, p. 51.

anastomoses are under the control of the vasomotor nerves, and their function is to divert the flow of blood from the arteries rapidly and directly to the veins. For this reason Sucquet termed them *canaux derivatifs*. In the event that the pressure in the arteries or veins becomes high, these anastomoses reestablish the normal pressure by opening communications between the two systems. In addition, by the same mechanism of opening and closing, they regulate both the local and the general temperature.

2. The arteriovenous anastomoses of the second group are the direct anastomoses between arteries and veins of large caliber. In man they are present before birth. In adults they are rare and are considered vascular anomalies.

The normal organs from which the glomal tumors spring were first described by Sucquet in 1862, after he had made special injections in various parts of the body. He described peculiar vessels in certain regions of the palm and sole, especially in the median zones of the ventral surfaces and in the fingers and toes (nail-beds especially). These appeared to be larger than the ordinary capillaries, and they opened directly into the neighboring veins without forming the usual capillary network. These structures were visible under low magnification.

Some investigators (Mueller [15] and others) tried to show that Sucquet was wrong, but Hyrtl [16] and Hoyer [17] in 1877 carried the studies of arteriovenous anastomoses farther and found these structures in the ears of rabbits, cats and dogs; he also demonstrated them in the snout, lips, toes of the forelimbs and hindlimbs, and tail. He did not find them in the skin of the head, knee or elbow of man. Grosser [18] in 1902 found them in man and many mammals but not in reptiles. Clara found them numerous and well developed in birds. Their absence in reptiles is readily understood when one considers that one of the functions is that of regulating the temperature. The reptiles belong to the class of poikilothermal animals in which the body temperature varies through a great range (changing with the surrounding temperature). This is too great a range to be controlled by such a delicate mechanism, or thermostat; in the birds and mammals, the warm-blooded type, the

15. Mueller, H.: Würzb. Naturwissenschaften Ztschr. **3**:168, 1863.

16. Hyrtl, J.: Denkschr. d. k. Akad. d. Wissensch. Math-naturw. Klasse, Vienna, 1864, vol. 22; Nat. Hist. Rev. **1**:99, 1862; cited by Grosser.[18]

17. Hoyer, H.: Arch. f. mikr. Anat. **13**:603, 1877.

18. Grosser, O.: Arch. f. mikr. Anat. **60**:191, 1902.

range of variation in temperature is small, and such an organ is necessary to prevent the temperature from going too low or too high.

In bats, Grosser [19] found the structure of the vessel wall in the region of the anastomosis to be like that observed in the vessels of the marrow in the terminal phalanx. In summing up the results of his studies, he stated that the chief characteristics of these anastomoses are the presence of longitudinal smooth muscle internal to the circular layer, the vanishing of the internal elastic lamina in the arterioles and the occurrence of large veins and venules and even of cavernous plexuses in the neighborhood of the anastomoses. The longitudinal muscle makes it easier for the circular muscle, in contracting, to close the lumen of the vessel; also, in contracting, the longitudinal muscle will stretch or distend the connecting vessel and thereby narrow the lumen. He noted also that in the anastomoses in the ball of the finger capillaries were present. While the anastomoses in the bat resemble those of higher mammals, they are not quite so characteristic. Grosser's wax constructions were excellent.

Recently Popoff [20] made a careful study of the normal glomus by means of serial sections parallel to the surface of the skin; the sections were also utilized in the preparation of wax reconstructions. His investigations were carried out on human material ranging in age from 4½ month old fetuses to adults. While other investigators used intravascular injections of colored substances, Popoff carried out his investigations on fresh surgical material without injections or colored materials. When fixatives were employed, Bouin's solution [21] was usually chosen.

A digital glomus is formed by the branches of a preterminal artery, and the zone of arteriovenous anastomosis is found a little deeper than the web of the subcutaneous plexus. The principal type of anastomosis is seen in the pulp of the fingers.

A glomus may be said to consist of (*a*) an afferent arteriole, (*b*) an anastomotic vessel, (*c*) a primary collecting vein, (*d*) intraglomar reticulum and (*e*) a capsular portion.

(*a*) The afferent artery arises in branches that run in the subcutaneous tissue parallel to the skin surface. On reaching the inner zone of the stratum reticulare, each divides into two branches, of which the smaller is concerned in the formation of the arteriovenous anastomosis. This branch is really the afferent glomal arteriole; it divides

19. Grosser, O.: Anat. Hefte **17**:205, 1901.

20. Popoff, N. W.: Arch. Path. **18**:295, 1934.

21. The solution is made up as follows: Solution of formaldehyde U. S. P., 500 cc.; distilled water, 1,500 cc.; glacial acetic acid, 100 cc.; trinitrophenol to saturation.

into several branches, each of which continues as an anastomotic vessel (Sucquet-Hoyer canal). Just before the glomal arteriole becomes the canal it gives off several small arterioles; these form a capillary plexus in the neuroreticular tissue for the supply of the wall of the canal, the reticulum and the nerves of the periglomal zone.

(*b*) The anastomotic vessel (the Sucquet-Hoyer canal) becomes twisted and crooked in its course and undergoes peculiar changes; the internal elastic lamina ceases abruptly at the beginning of the coils, and, just were the canal begins, the collagenous and elastic layer, just under the endothelium, assumes a ruffle-like arrangement that is characteristic. This must be of physiologic significance, as certain pathologic changes are first noted here. The lumen of the canal becomes irregular owing to the fact that the endothelium becomes altered. The endothelial cells become cuboid and may form two or three rows. The internal elastic lamina disappears, and the endothelial cells rest on the smooth muscle tissue. In addition to the ordinary circular layer there are longitudinally disposed smooth muscle fibers just beneath the endothelium. These longitudinal fibers are so arranged as to form longitudinal folds of the endothelium, giving the internal surface of the vessel a corrugated appearance. The endothelium and the muscle cells seem to mix, but they are differentiated from each other by the larger size and the deeper stain of the endothelial cells. These elevations correspond to the region of origin of the canal and the periglomal arterioles, and Popoff surmised that they function in such a manner as to guide the blood into the canal and periglomal arterioles. Similar longitudinal shafts are said to be located external to the muscle layer of the media; Popoff, however, stated that no true separate layers of circular and longitudinal muscle are to be found in the canal. The longitudinal muscle fibers, however, are shorter and thicker than those of the circular layer, and their nuclei are oval instead of rod-shaped. Amid the muscle cells are said to be some large clear epithelioid glomal cells with oval or globular nuclei poor in chromatin. These cells have been variously described as immature or embryonic muscle cells, or myoblasts, while Krompecher considered them angioblasts that become elastoblasts and form elastic tissue. The cytoplasm of these cells is clear or vacuolar and does not respond readily to ordinary plasmatic stains; it is free from myofibrils and does not respond to stains for glycogen, fat or mucin. Spreading between these elements are some anastomosing elements with elongated nuclei and transparent cytoplasm, devoid of myofibrils. At the beginning of the canal, the muscle coat becomes narrower and more compact. The venous end of the canal is characterized by its thin wall, containing few muscle fibers, by the disappearance of the nerve network and by an abundance of elastic

tissue in its outer coat. This convoluted anastomotic canal is called the Sucquet-Hoyer canal. The measurements are given as follows:

	Nail-Bed	Ball of the Finger
Diameter (outside), microns	55-60	90-100
Lumen, microns	18-22	10- 30

The lumen changes during life within wide limits and may be entirely occluded.

(*c*) The primary collecting vein is the vein into which the anastomotic canal empties. It is thin walled, is poor in or devoid of muscle tissue and shows nothing that is different from the ordinary venule. In reconstructions it seems that the primary collecting vein has the shape of a broad ruffle or cape encircling the glomus and forms a voluminous receptaculum with a highly developed surface area. These veins are rich in elastic tissue but poor in muscle elements, and they empty into the subpapillary plexus and through this into the deeper veins of the skin. They are provided with valves.

The width of the primary collecting vein when empty is almost that of an ordinary capillary, that is, from 8 to 10 microns. On longitudinal section the vein has the appearance of a capillary slit from 200 to 300 microns in length.

(*d*) The entire muscle coat is surrounded by a wide clear zone consisting of a delicate collagenous reticulum—the intraglomar reticulum —which contains numerous amyelinated nerve fibers; these form a dense network of neurofibrils around the muscle wall of the canal, which is readily demonstrated by the silver stain. Special nerve endings are apparently absent. There seems to be little intermuscular tissue, and the internal and external elastic coats are not developed. This clear zone may be a neuromuscular mechanism for the control of the function of the canal. In this zone is the main part of the capillary network formed by the periglomal arterioles. The blood from this source is collected by vessels that carry it to the subpapillary plexus and not to the primary collecting vein of the glomus.

(*e*) The capsule, that is, the superficial portion of the glomus, consists of a delicate reticulum of collagenous tissue in which are many amyelinated nerve fibers, which are demonstrable by various methods of staining, as methylene blue and silver. Peripherally this tissue is reenforced by lamellated coarse collagenous tissue, in the meshes of which are found the collecting veins. This coarser tissue in the form of a weak capsule serves to separate the glomus from the surrounding tissues.

The number of glomuses to the square centimeter varies in the different parts of the body; it also varies with the different investigators.

All parts of the body have not been investigated in this regard. Grant and Bland [22] gave the following numbers:

Index finger	
Nail-bed	501
Tip	236
Palmar surface of the third phalanx	150
Palmar surface of the second phalanx	20
Palmar surface of the first phalanx	93
Thenar eminence	113
Hypothenar eminence	96
Second toe	
Nail-bed	593
Pad	293
Sole near nail-bed	197

The numbers obtained by Popoff in his studies are quite different. His investigations were carried out on the ventral and lateral (both) surfaces, nail-bed and nail matrix. He stated that the results were influenced by a number of factors, of which age and pathologic processes were the most important. In fetuses ranging in age from 4½ months to birth these canals were absent, although sweat glands, nerve trunks and tactile corpuscles were well developed. In persons of 60 years the glomuses begin to decrease in number, and this decrease progresses with advance of age. His findings are as follows:

Great toe (at 20 years)	
Ventral surface	18
Lateral surface	10
Nail-bed	24
Matrix	12

The figures obtained from corresponding parts of the thumb are approximately the same. The counts are strikingly lower than those of Grant and Bland; they were taken from serial sections and so are inclined to be the proper numbers.

As to the dimensions of the canals, Grosser gave the following:

	Nail-Bed	Finger Pulp
Diameter (outside), microns	55- 65	18-22
Lumen, microns	90-150	10-30

Popoff included the periglomal clear zone as a part of the canal. His measurements are as follows:

	Pad of Toe	Nail-Bed
Diameter (outside), microns	120-220	60-150

NERVES AND FUNCTION

The digital glomuses, or arteriovenous anastomoses, are considered to be, by virtue of their muscular structure, mechanisms (manometers, thermostats, regulators) for the control of the blood pressure, interstitial pressure and local temperature in those parts of the body in

22. Grant, R. T., and Bland, E. F.: Heart **15**:385, 1931.

which they are located. By the play of musculomotor reflexes they regulate the blood pressure of the terminal arteries.

A glomus may be considered as serving at least two functions, local and general. If a digit is exposed to cold, the local temperature is not only maintained but even increased through action of the glomuses. This is brought about by diverting the blood from the capillaries and rushing it through the anastomoses into the collecting veins, which have a highly developed surface area. Again, if the pressure becomes abnormally high, these anastomotic by-passes divert the arterial blood, thus relieving the peripheral arterial system of the extra burden. Also, if the peripheral veins and venules are unable to cope with the influx of blood, the anastomosis, by sending a part of the blood directly into the deep veins, helps to correct the local disturbances in the venous circulation.

Lewis and Pickering [23] and Grant and Bland [22] showed experimentally, by the use of physical, mechanical and chemical means, that this system is important in the regulation of local and general temperatures.

The absence of this system in the fetus seems to indicate that it is not essential during gestation. The fetus in utero is subjected to little change in temperature, and what there is can readily be adjusted and controlled by the mother. It is known that the heat-regulating mechanism is one of the last developed in the premature infant. This may explain why it is necessary to keep such an infant under conditions that will permit of little variation in the surrounding temperature (incubator). The development of this system takes place during the first months of postnatal life (normal term). It is known that the body temperature of the normal infant shows marked fluctuations with a tendency to hypothermia, indicating too rapid radiation or loss of heat. This is due to various causes: lack of development of the glomal system, incomplete development of the nerve system (especially the respiratory center), weak cardiac action, too rapid radiation of heat, insufficient combustion of oxygen and improper metabolism.

The glomuses possibly explain the differences in degrees between frosted and frozen extremities. In cases of mild frost bite, no injury results because these structures have not been materially affected. In cases of moderate chilblain, these organs have been somewhat affected, so that they do not always function properly; some may have been disorganized entirely, so that the remainder cannot give complete control of the temperature and pressure. In cases in which amputation is necessary, all of these organs may have been destroyed, so that when

23. Lewis, D., and Pickering, G. W.: Heart **16**:33, 1931.

the part has been thawed, the loss of function of these structures and the damage to the surrounding tissues make amputation imperative.

The frequent coexistence of pacinian bodies with the anastomoses seems to substantiate the belief that these organs have to do with the control of the temperature and blood pressure. There is experimental evidence in favor of the idea that the function of the pacinian body is that of a regulator of the blood pressure; stimuli that this body receives produce reflex contraction or dilatation of the associated vessels. These corpuscles are normally elliptic, but with an increase in blood pressure or with drenching or saturation of the tissues changes occur so that the corpuscles become round.

A digital glomus could also be considered an organ of Ruffini, which is found at the junction of the vessels making up an arteriovenous anastomosis. Each vessel is surrounded by a thin layer of neural tissue which is continuous with the expansions of the sympathetic nerves of the veins as well as with those of the arterioles. The organs of Ruffini serve the purpose of regulating the blood pressure; they keep the capillary pressure as well as the local temperature constant; in addition, they play a part in tactile sensation.

The digital glomus is analogous to the segmental glomeruli discovered by Arnold in the tails of certain monkeys and in dogs. He was able to show also their analogy to the glomus coccygeum.

Efforts to reproduce the anastomoses artificially have been made by Carrel and Guthrie.[24] Their experiments demonstrated that mechanical conditions created by reversing the circulation were met with unusual adaptability on the part of the arteries and veins. In arteriovenous fistulas of the cardiac wall the vein becomes dilated and altered so as to resemble an artery in structure, as was found by Reid[25] and confirmed by Popoff. It also was noted that in a fistula of long duration the artery became dilated proximal to the fistula (spindle-shaped dilatation).

RECAPITULATION OF KNOWLEDGE OF THE NORMAL GLOMUS

A normal glomus is a distinct structural unit or organ consisting of (*a*) an afferent arteriole, (*b*) a Sucquet-Hoyer canal, (*c*) a primary collecting vein, (*d*) intraglomar reticulum and (*e*) a capsular portion.

It controls the arteriovenous circulation of that part of the body in which it occurs and regulates the local and general temperature of the body. The absence of this system at birth explains the trophic and thermal disturbances that are present at that time; continuance of such

24. Carrel, A., and Guthrie, C.: Compt. rend. Soc. de biol. **58**:780, 1906.
25. Reid, M.: Bull. Johns Hopkins Hosp. **31**:43, 1920.

disturbances after a reasonable length of time may be attributed to lack of or improper development of this system.

The absence of glomuses in supernumerary digits has not been conclusively demonstrated, as the tissue examined was from stillborn infants or from infants aged up to 2 weeks. This system does not develop so soon as that but develops during the early months of postnatal life.

In advanced age the glomuses undergo atrophy and also become reduced in number.

Glomuses are said to be unstable and sensitive to various influences, showing both regressive and progressive changes. Changes of the latter type are illustrated in the event of collapse of and loss of a canal: a new canal develops from one of the periglomal arterioles.

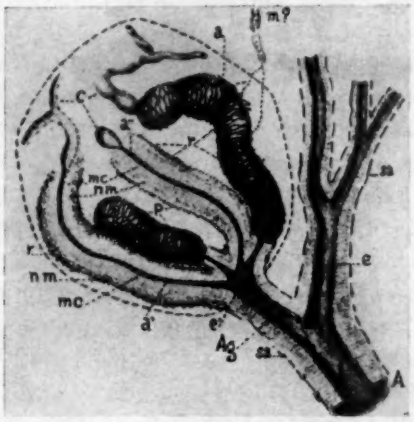

Fig. 1.—Schematic reconstruction of a subcutaneous glomus after Masson[1]: *A*, dermal artery; *Ag*, glomal arteriole forming four anastomotic channels (canals); *e*, elastica of *Ag*, which disappears when the arteriole becomes the canal (Sucquet-Hoyer); *mc*, smooth muscle tissue, which is increased, and endothelium, which is modified; *nm*, neuromuscular layer containing a network of amyelinated nerve fibers (according to Masson, the cells here are ganglion cells); *m?*, hypothetic connection between amyelinated nerve fiber and the perivascular network; *c*, collecting veins ultimately emptying into the subpapillary venous plexus; *a*, arteriole with rich perivascular network closely resembling an organ of Ruffini; *a'*, endothial layer; *a''*, venous end of the anastomotic canal; *r*, section of the reticular layer.

HISTOLOGY OF GLOMAL TUMORS

In general histologic appearance the glomal tumor bears a striking resemblance in structure to the glomus coccygeum. The various elements are so much alike that it seems that one is dealing with a very much enlarged gland of Luschka. The tumor arises, without doubt, from one or other of certain little organs of the terminal arterioles, especially the arteriovenous anastomosis of the digital pulp.

The fact that accidental hyperplasia of the digital glomus confers on it the structure of a gigantic glomus coccygeum confirms, it seems, the homology to the latter so distinct little organ; hence the belief that the nerve elements play an important part in its histologic make-up; this makes one believe further that the normal glomus, both digital and coccygeal, possesses a definite and special innervation which is poorly understood.

The tumor is surrounded by several layers of collagenous tissue which, according to some investigators, do not form a definite capsule; others, however, state that a capsule exists. In any case the tissue seems sufficient to set the mass off from the surrounding structures. Bailey [8] considered this the result of condensation through pressure rather than a true capsule. At one pole of the tumor there is usually a large arteriole representing the nutrient vessel; this is enveloped by a network of nonmedullated nerve fibers, which form a sort of true spread-out intracapsular neuroma. From this principal neuroma, little nerve fibers pass to the interstices of the capsule. In addition to arterioles the capsule contains venous capillaries and little veins with extremely thin muscular walls. While the vessels of the glomal tumor usually lack the elastic lamina, some vessels show such a structure. As the latter vessels do not possess any of the characteristics of the true glomal vessels, Bailey considers them branches of the ordinary periglomal arterioles or of extraglomic origin. The tumor itself presents several aspects which are quite different depending on whether one examines the capsular region, the region in the center or the intermediate areas.

The cortical or subcapsular portion is infiltrated by a number of blood sinuses lined with continuous endothelium; these channels anastomose freely. The spaces between the anastomosing sinuses are filled with cells of all forms, especially elongated and polyhedral cells; these are of an epithelioid nature and are surrounded and separated from one another by a collagenous network. While in certain places the collagen comes in contact with the endothelium, in most regions thick masses of cells intervene. The vessels to a great extent are almost completely surrounded by a multistratified cellular layer. Deeper down the collagenous partitions become more spread out and less clearly fibrillar. Some mucoid material is found separating the cells from one another.

In the center of the tumor the collagenous partitions are still larger, hyalinized and farther removed from the cellular masses. The spread-out vessels are in contact with the irregular cords of cells that are set off here and there as little lobules by the reticulum. These areas are infiltrated with the hyaline material. This mucoid or hyaline substance is quite abundant, as though mucoid degeneration had occurred.

According to Barre and Masson,[26] there are two types of vessels in glomal tumors: (*a*) those in which the epithelioid cells are in contact with the endothelium of the blood vessels; (*b*) those in which the endothelial cells of the blood vessels rest on a collagenous membrane that is supported by two layers of smooth muscle fibers, circularly arranged. Peripheral to these muscle fibers there are some larger, slightly elongated elements with globular nuclei; the cytoplasm is faintly acidophilic. These cells merge into the surrounding epithelioid (glomal) cells. These intermediate cells are apparently the cells to which some investigators refer as the "gradation cells" between the epithelioid and muscle cells.

Other investigators (Paulian, Popescu and Marinesco-Slatina[27]) have found that the walls of the blood vessels are peculiar. In some of the channels which they observed, the lining endothelial cells were distinctly cuboid and rested on a collagenous membrane. Certain vessels were surrounded by circular muscle tissue that passed by a series of imperceptible gradations into a broad zone of epithelioid (glomal) cells. Other vessels were separated from the surrounding cells by fine connective tissue fibers; the cellular elements were elongated structures with rounded nuclei and sharp nucleoli. The cytoplasm was clear and stained faintly with eosin. Scattered between these were small spindle-shaped cells with scant cytoplasm, darkly staining elongated nuclei and processes that streamed from both poles. Occasionally the cells were embedded in a homogeneous matrix that resembled that of hyaline cartilage. The cells were separated by a dense network of fine fibers that became continuous with the coarser strands between the vessels.

In some tumors the elastic tissue is abundant, while in others the quantity is small. Mason and Weil[28] have shown the abundance of argyrophil fibers; these and the myelinated and amyelinated nerve fibers may be readily demonstrated by the Perdrau and Davenport methods. They resemble those of the glomus coccygeum very closely. At the periphery of the tumor the nerves are large and prominent; they give rise to branches that enter the tumor and redivide, the terminal branches ending around the glomal cells. These terminations are composed of several fine filaments that spread out on the glomal cells with or without terminal bulbs. Although aniline dyes bring out the nerve plexuses very well, they do not show the exact method of termination of the fibers, according to Bailey.[3]

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26. Barre, J. A., and Masson, P.: Bull. Soc. franç. de dermat. et syph. **31**: 148, 1924.

27. Paulian, D. E.; Popescu, S., and Marinesco-Slatina, D.: Ann. d'anat. path. **10**:271, 1933.

28. Mason, M. L., and Weil, A.: Surg., Gynec. & Obst. **58**:807, 1934.

The most important part of the tumor, however, is what might be termed the parenchyma; this consists of cells that are peculiar in appearance and that have neither a definite nor a constant type of arrangement. They are found distributed in the form of cords, masses or groups that vary in size. They may be packed about the vessels in a compact mass which, however, is seen to be broken into groups of two or three cells when sections are stained by Mallory's or Hortega's methods; under these conditions the cell boundaries are indistinct, There may be large groups of cells independent of blood vessels, or the glomal cells may be seen singly or in small groups embedded in a homogeneous material that responds faintly to the plasmatic stains. This mucoid material is said to be the result of degeneration of the glomal cells. The glomal cells practically fill the spaces between the blood vessels. At the basal area, or pole, they are scattered or diffusely arranged. The arrangement and appearance vary at different depths.

These glomal cells are apparently epithelioid, though some object to this term. They are, for the most part, large polyhedral elements with palely staining cytoplasm and fairly deeply staining nuclei. The cytoplasm is said to be brownish after fixation in solutions containing chromium salts. The chromatin is in the form of granules. A few of these granules are black after staining by the Cajal method. No mitoses have been seen.

The large cells are considered by some to be immature and undeveloped embryonic smooth muscle cells (myoblasts); some investigators claim that they have seen the various stages of evolution from the indifferent cell to the fully developed smooth muscle fiber. Bailey considered the glomal cells as modified smooth muscle elements with abundant nerve connections. He surmised that in the formation of a glomal tumor the smooth muscle cells lose all of their myofibrils, while the intervening supportive tissue becomes increased and coarser. Next, the periglomal nerves invade the tumor, terminating about the modified muscle cells, with the formation of nerve endings. These two processes, although independent of each other, result in the formation of glomal cells.

If these two processes are independent of each other, this seems a rather haphazard method for the formation of glomal cells and one that would more often fail than succeed. Again, the changes as described are retrogressive and not progressive; this is diametrically opposed to the view that the epithelioid (glomal) cells become altered into smooth muscle fibers (representing, thus, myoblasts). Previous authors have asserted that the latter changes occur and not the reverse.

These epithelioid cells resemble very closely those of the glomus coccygeum. All investigators are agreed on this point. In their inter-

pretations as to what the epithelioid cells represent, they are at variance. Krompecher,[29] in his recent work on the development of the glomus coccygeum, confirmed the work of Stoerk,[30] Clara,[31] von Schumacher [32] and Benninghoff.[29] These investigators stated that the epithelioid cells of the glomus coccygeum are angioblasts and not myoblasts. They are said to produce the elastic network that envelops the cells of the gland of Luschka (elastoblasts) and also to form a fill-in substance between the endothelium and the media of the arteriole. These investigators asserted positively that these cells do not change into smooth muscle cells.

Glomal tumors are, in a sense, angioma with an unusual amount of neural and of smooth muscle tissue, hence the name of angioneuromyoma. Here, however, the neural tissue is secondary, being present as a result of the overdevelopment of the blood vessels and the muscle tissue. The characteristic cells present, whether they are to be considered myoblasts or angioblasts, are of mesodermal origin, so that it seems that this tumor is a mesodermal rather than an ectodermal derivative.

That the neural tissue is increased is granted, but it seems that this increase is secondary rather than primary. Vascular tumors are exceedingly painful, and this points more to a mesodermal origin.

REPORT OF CASES

CASE 1.—A woman aged 26, a patient of Dr. L. D. Englerth, showed a small lump on the radial side of the left thumb, toward the palmar surface of the distal phalanx. This had appeared approximately ten years earlier. It gradually increased in size, was always sore and toward the end became exceedingly painful. The pain was so severe at times that the patient could not sleep. Three years after the operation, the area showed a slight scar, and there was slight numbness in the end of the finger, but no pain.

Several sections through the middle of the tumor were submitted for study. Sections from the remainder of the block were stained with various stains.

The capsule proper consisted of collagenous tissue and contained numerous small blood vessels. At irregular intervals trabeculae extended centrally; the larger ones contained medium-sized arterioles and thin-walled venules. The trabeculae terminated in many coarse and fine fibers that formed an irregular network, most of which responded well to the special collagenous stains. Reticulum cells seemed to be numerous along the edges of the cell nests. Many fibers around the reticulum cells did not respond to the collagenous stains and were probably reticulum fibers. Within the meshwork were found the parenchymal cells and many sinus-like dilated blood channels. These spaces were largest at the periphery of the section. The wall of the channels consisted in the main of a single layer of endothelial

29. Krompecher, S.: Verhandl. d. anat. Gesellsch. **41**:176, 1932.

30. Cited by Krompecher.[29]

31. Clara, M.: Ergebn. d. Anat. u. Entwcklngsgesch. **27**:246, 1927.

32. von Schumacher, S.: Arch. f. mikr. Anat. **71**:58, 1908.

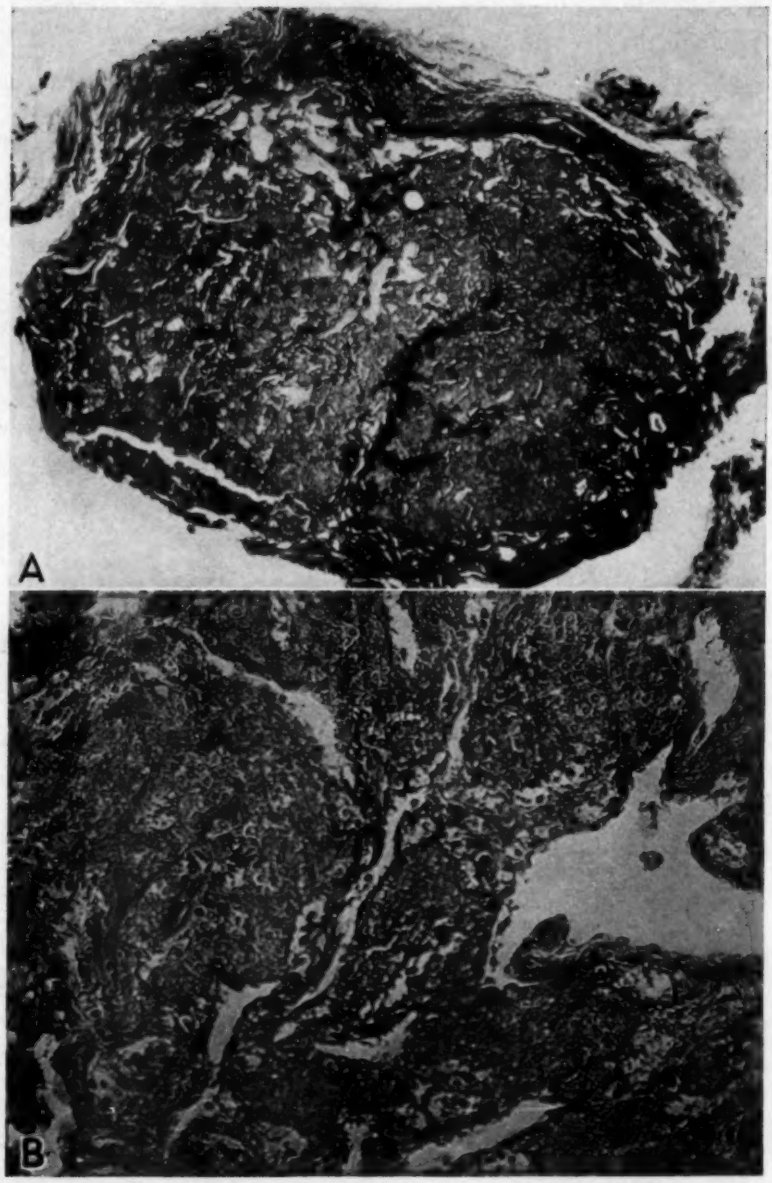

Fig. 2.—*A*, section of the tumor in case 1; × 10. The section shows the well developed capsule of the tumor and the vascular character of the parenchyma. *B*, a portion of the tumor showing a few of the vascular channels and the glomal cells in compact masses; × 100.

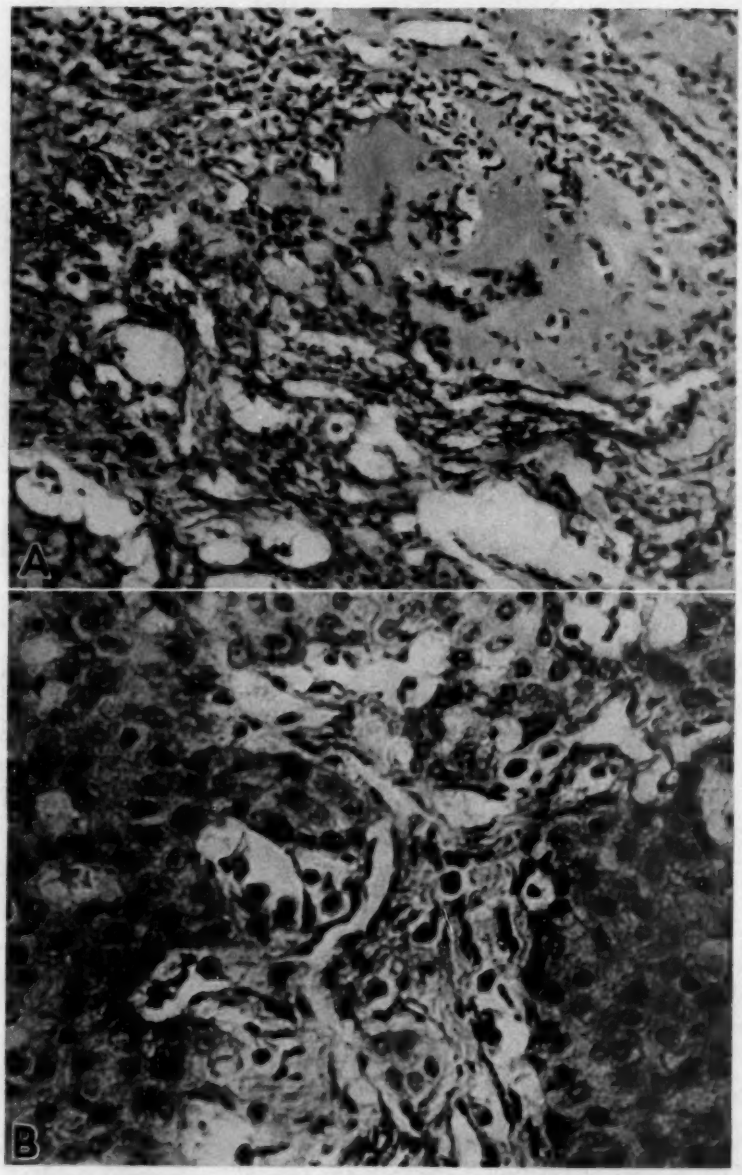

Fig. 3.—*A*, another portion of the tumor, showing a looser arrangement of the supportive tissue, a few glomal cells and an abundance of mucoid material; × 200. The latter is represented by the grayish shading in the spaces. *B*, the cell boundaries of the glomal cells are indistinct, but the nuclei are clear. The endothelial cells of the vascular channels stand out clearly. The center of the field shows some extravascular cells with prominent deeply staining nuclei. The entire cell is about the size of the nucleus of the glomal cell. These small cells occur singly or in small groups well separated from one another; × 400.

cells, chiefly unsupported; some of the smaller channels contained a few cuboid cells while a few small vessels showed some cells apparently rounding off (endothelium). In only a few instances were smooth muscle fibers noted in connection with vessels.

Variable-sized masses of apparently homogeneous material were scattered throughout the section; this material was hyalin in appearance and apparently mucoid in nature. Some of the masses, however, showed a fibrillar structure and so may have been prefibrillar material. This material responded somewhat to mucicarmin stain and was considered to be the result of degenerative changes.

The most interesting part of the tumor, however, was what might be termed the parenchyma; this consisted of cells that were peculiar in appearance and had no definite or constant arrangement. They were arranged in cords, masses or groups of different sizes. They practically filled the spaces between the blood vessels and for the most part were closely packed. They were apparently epithelioid in nature. They were large polyhedral elements with indistinct cell boundaries but had lightly stained clear nuclei, in which the chromatin was granular. These glomal cells resembled those of the glomus coccygeum very closely.

Cells of a smaller type were present. They stood out sharply in contrast to the glomal cells; they were smaller, the cell boundaries were sharp and clear, and the nuclei were deeply stained owing to the large amount of chromatin present. These cells were comparatively few and occurred singly or in small groups of not over 6 cells. They were chiefly in the extravascular spaces and seemed to be rounded-off endothelial cells or polyblasts. They stood out especially in sections stained with alkaline eosin-methylene blue. Cuboid endothelial cells of the blood vesels such as those mentioned by Paulian, Popescu and Marinesco-Slatina [27] and muscle fibers and the transition from epithelioid cells to muscle cells as mentioned by the same investigators were not observed.

Staining of sections with Weigert's elastica stain gave negative results. With methylene blue small groups of stellate cells were to be seen in relation with the smaller blood vessels; these were probably sympathetic ganglion cells.

The diagnosis was glomal tumor of the thumb.

Case 2.—The tumor was from a woman aged 30 years, a patient of Dr. H. K. Seelaus. It began five years earlier with a tender spot on the palmar side of the middle finger. It did not bother her except when it was hit. As time went on, however, when the tumor was struck against anything, the pain would continue for a while. About the same time she noticed that it became sensitive to cold. About two years before operation there were times when she got a sharp pain at the end of the finger, lasting for a minute or two; on some days the attacks became more frequent than on others. Later she experienced pain shooting up the arm, which at times was so severe as to awaken her from sleep. All sorts of treatment was tried, and many physicians were consulted, but she experienced no relief.

Pathologic Report (Dr. B. L. Crawford).—The specimen consisted of a small nodule of tissue that measured 0.5 cm. in its greatest diameter. Fixation was obtained with dilute solution of formaldehyde U. S. P. plus alcohol, and a rapid technic was followed.

Examination of sections revealed that the nodule was composed of very cellular tissue in which there were numerous small endothelial-lined spaces. These small spaces were surrounded by collections of rather large cells that had granular cytoplasm and relatively large, deeply staining nuclei. These cells formed solid clusters around the spaces, and between the clusters of cells there

was considerable connective tissue of loose texture that seemed edematous and also contained blood vessels. No definite capsule was observed.

Because of the location of the growth and the presence of a large number of vessel and endothelial-lined spaces and the peculiar large cells, the growth is considered to be of the glomal type.

The diagnosis was glomal tumor of the finger.

CASE 3.—This case was observed by Dr. Thomas Shallow. About four years earlier the patient, a woman aged 35, felt a small mass in the left arm, which at first was not tender or painful. She had no treatment until about a year before operation, when she noticed that the mass was increasing, slowly at first; six months before operation it began to grow more rapidly. Coincident with this it became very painful and tender to the touch.

Pathologic Report (Dr. B. L. Crawford).—The specimen consisted of a soft nodule of friable reddish tissue, which measured 3 by 3 by 2 cm. and weighed approximately 10 Gm. The external surface was smooth, and the nodule seemed encapsulated. On section the tissue was red in some areas and yellowish in others. It seemed to contain small cystic areas which were filled with a dark reddish fluid. It was fixed in solution of formaldehyde U. S. P., and sections were prepared by a routine technic.

The sections revealed that the nodule was composed of a very cellular tissue, in which there were numerous small vessels and rather large spaces, many of which were filled with blood. These spaces seemed to be lined with endothelial cells. The cellular tissue was made up of connective tissue, in which there were numerous collections of rather large cells with large deeply staining nuclei; some of these cells formed small clusters, and others seemed to be arranged around small spaces. At the margins of the tissues there seemed to be small collections of muscle fibers, suggested by long rod-shaped nuclei. This somewhat palisade-like arrangement of the cells in the connective tissue gave them a resemblance to nerve cells, but no definite nerve fibers were observed. The nodule seemed to possess a definite fibrous capsule, in which there were numerous vessels. In some areas of the tumor the connective tissue was of loose texture and seemed edematous, and considerable extravasated blood was present.

Because of the location of the tumor in the arm, the presence of blood spaces and the intervention of stroma tissue between the cells the lesion was considered to be of the glomal type.

The diagnosis was glomal tumor of the subcutaneous tissue of the arm.

CASE 4.—This tumor was from a white woman 22 years of age, a patient of Dr. Edward McLaughlin. She had it for five years and recalls no trauma that may have caused it. It was located on the medial side of the base of the distal phalanx of the thumb, at the base of the nail. It was bluish, slightly oval, and measured about 3.5 by 5 mm. At operation it was punctured, but no fluid was liberated. It was tender to pressure but not to light touch. The pain and tenderness became increasingly severe. Local anesthesia sufficed at operation.

Pathologic Report.—The tumor was surrounded by a distinct capsule of collagenous tissue, which contained numerous blood vessels, notably thick-walled arterioles. The collagenous tissue of the capsule responded well to the special stains. Within the tumor mass the collagenous tissue consisted of fine fibrils, separating the parenchymal cells from one another.

The vascularity of the tumor was well marked, as the thin-walled, endothelial-lined blood channels were numerous and large. Mucoid material responding to the hematoxylin stain was very abundant throughout the sections, especially near

the capsule. While some of it was homogeneous, most of it seemed somewhat fibrillar. The masses of mucoid material were largest in the large group of parenchymal cells.

The parenchyma consisted of small and large groups of glomal cells; these filled the spaces between the blood channels completely. The cell boundaries were distinct and the nuclei large and well stained. The cytoplasm was rather homogeneous but stained lightly.

Several of the blood vessels showed thickened endothelial cells with oval or circular nuclei. Rounded-off endothelial cells or polyblasts such as those encountered in case 1 were not noted.

Numerous fine and coarse elastic fibers were found not only in the capsule but throughout the tumor mass, especially in the neighborhood of the blood vessels; they were present in the mucoid areas also.

The diagnosis was glomal tumor of the thumb.

At operation in both cases 2 and 3 there was shown what is apparently a characteristic of such cases, namely, that it is difficult to anesthetize the area from which the tumor is to be removed. In fact, in case 3 it was necessary to finish the operation under nitrous oxide and oxygen anesthesia. To date none of the patients has had a recurrence of the lesion, and all three are considered cured.

SUMMARY

The glomal tumor represents a pathologic alteration of a normal histologic structure, the glomus. The tumor was formerly classified under various names. While it may occur anywhere in the body, it seems to occur more frequently in the upper extremities, especially in the fingers. It occurs more frequently in females and affects the left side more frequently than the right side. It occurs between the ages of 20 and 80, although one was reported in a child of 6 years. Records show that the shortest duration was six weeks and the longest thirty-seven years.

The tumor is always small, and fortunately its growth is slow. The slowness of growth and small size are readily understood in the tumor that is subungual. It is benign, and the prognosis is favorable. Excision effects a permanent cure, as no recurrence has ever been reported. The tumor is usually painful on the slightest pressure, especially if it is subungual. At times even the slight pressure of the clothing causes considerable pain. Again, the pain may be constant or in crises.

PRODUCTION OF COLITIS IN THE DOG WITH STAPHYLOCOCCUS TOXIN

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Necroses and hemorrhages in the mucosa of the gastro-intestinal tract may follow intravenous [1] and oral [2] administration of staphylococcus toxin. Rigdon and Leff [3] recently described a hemorrhagic and necrotic lesion in the intestinal mucosa of each of two children, one of whom had otitis media, mastoiditis and meningitis, and the second, osteomyelitis of the tibia and acute pericarditis. Staphylococcus aureus was present in both cases. Staphylococcus toxin was considered as the etiologic agent in the production of the intestinal lesions.

Previously described lesions in the gastro-intestinal tract were usually found in animals that died following intravenous injection of staphylococcus toxin. In the present experiment staphylococcus toxin was injected locally into the colons of dogs. The animals were killed at intervals in order to study the development and the process of healing of the ulcers.

MATERIAL AND METHODS

The animals used in this study were twelve normal dogs weighing from 4.5 to 12 kilograms. The toxin was prepared by growing a hemolytic strain of Staphylococcus aureus in veal infusion broth. The method of preparing this toxin is described elsewhere.[4] A 0.02 cc. volume of this toxin produced complete hemolysis of 2 cc. of a 1 per cent suspension of rabbits' red blood cells in physiologic solution of sodium chloride, and 0.1 cc. injected intracutaneously into a rabbit produced an area of necrosis measuring 2 by 4 cm.

Each dog was anesthetized by an intravenous injection of 30 mg. per kilogram of pentobarbital sodium dissolved in physiologic solution of sodium chloride. The abdomen was opened and the colon brought out through the incision. The toxin was injected through the wall of the colon into the submucosa and mucosa. A 26 gage needle was used. The volumes of toxin given to the different dogs varied from 4 to 6 cc. The amounts injected into the different areas varied from 0.1 to 0.5 cc. Approximately one third to one half of the total length of the colon in each dog received injections. The points of injection of the toxin were usually from 0.5 to 2 cm. apart. When the foregoing procedure was completed, the colon was replaced in the abdominal cavity, and the incision was closed.

From the Department of Pathology, Vanderbilt University Medical School.

1. Rigdon, R. H.: Arch. Path. **20**:201, 1935.
2. Borthwick, G. R.: Brit. J. Exper. Path. **14**:236, 1933.
3. Rigdon, R. H., and Leff, W. A.: Arch. Path. **21**:298, 1936.
4. Rigdon, R. H.: J. Infect. Dis. **60**:25, 1937.

The animals were given all the sweet milk, scraps of bread, dog biscuits [5] and water which they desired during the experiment.

The dogs were put to death at the intervals shown in the table. A complete autopsy was performed immediately, and the tissues were fixed in a 4 per cent formaldehyde solution. Histologic sections were stained with hematoxylin and eosin. Serial sections were made through some of the ulcers.

Each of two dogs received an injection of 5 cc. of infusion broth in the colon for the control experiment. The same technic was used as described for the toxin.

All the animals apparently were in good health when put to death except one. This dog was moribund thirty hours after the operation.

CLINICAL OBSERVATIONS

The blood vessels in the serosa of the colon dilated very quickly following the first few injections of either the toxin or the infusion broth. The wall of the colon also contracted during this period. The dogs remained unconscious or semiconscious for from six to ten hours after the operation. During this period they had abdominal

Details of Experiment

Dog	Weight, Kg.	Amount of Toxin or Infusion Broth Injected into Colon, Cc.	Time Elapsing Before Dog Was Killed
22	10	6 Toxin	4 days
23	6	5 Toxin	7 days
24	10	5 Toxin	14 days
25	6	5 Toxin	Moribund after 30 hours, killed
40	5.5	5 Infusion broth	4 days
41	7.5	5 Infusion broth	10 days
42	6.5	5 Toxin	8 days
43	7.5	5 Toxin	8 days
44	9	5 Toxin	4 days
45	7.5	5 Toxin	24 hours
46	4.5	4 Toxin	3 days
47	5.0	4 Toxin	24 hours

cramps and tenesmus and often passed soft stools. After recovering from the anesthesia they took food and water and never appeared very sick. The stools were frequently liquid during the first seventy-two hours after operation. They were soft and often contained blood and mucus until the fifth day, at which time they became normal except those from dog 42. This animal showed mucus and blood in the stools on the eighth postoperative day.

The stools from the two dogs in the control group showed very little change from the normal after the first forty-eight hours.

PATHOLOGY

A majority of the animals showed evidence of bacterial infection in their abdominal wounds. Several of the dogs had localized peritonitis in the tissues adjacent to the incisions. Only one dog had generalized peritonitis. He was moribund thirty hours after the injection of the toxin and was killed.

The only lesions of interest in any of the dogs were located in the colon. Every animal receiving staphylococcus toxin showed evidence of injury to the mucosa.

5. The Purina Mills Company supplied the following analysis of the chow: 5 per cent fat, 23 per cent protein, 4 per cent fiber, 7 per cent ash, 7 per cent moisture and 54 per cent nitrogen-free extract.

The extent of the pathologic process varied in the different dogs. Twenty-four hours after the injection of the toxin, the wall of the colon was edematous and hemorrhagic. The mucosa was necrotic, and areas were sloughing (fig. 1*A*). The lumens of the glands in the mucosa were frequently filled with degenerated epithelial cells and débris (fig. 2*A*). The epithelial cells lining the glands showed all degrees of injury from cloudy swelling to coagulation necrosis.

The hemorrhagic discoloration and the edema in the colon were apparently considerably decreased by the second to the fourth postoperative day. Ulcers,

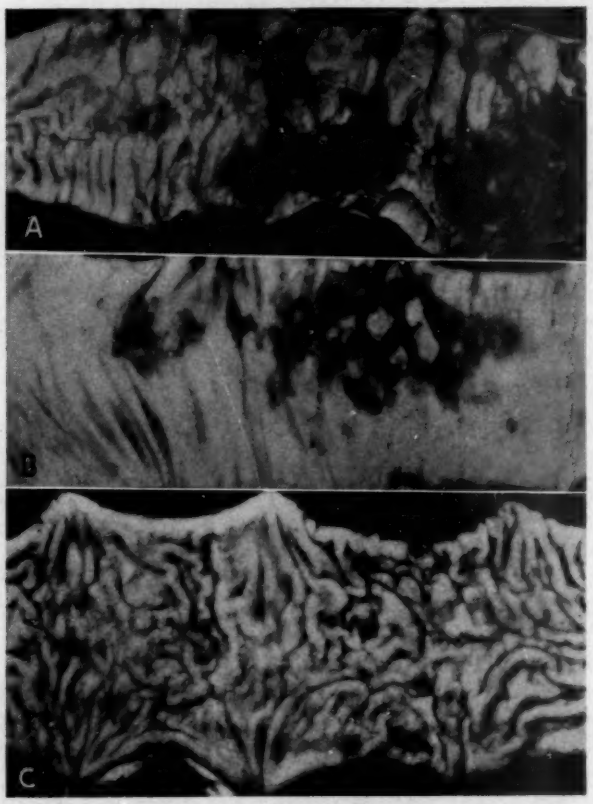

Fig. 1.—*A* (dog 45), hemorrhagic necrosis of the mucosa and edema of the wall of the colon twenty-four hours after 5 cc. of the staphylococcus toxin was injected directly into the colon. *B* (dog 44), ulceration of the mucosa of the colon four days after 5 cc. of staphylococcus toxin was injected directly into the colon. *C* (dog 43), foci of atrophy in the mucosa. These areas apparently represent completely healed ulcers. Staphylococcus toxin was injected directly into the colon of this dog eight days previously.

however, were present in the mucosa. The margin and base of each ulcer were irregular and the adjacent tissues were infiltrated with leukocytes (fig. 3*A*). Small areas of apparently viable mucosa were present in the centers of some of the larger ulcers (fig. 1*B*).

Regeneration of the mucosa apparently begins during the first twenty-four to thirty-six hours after the injection of the toxin. Cystlike spaces were present in the mucosa after the second or third day (fig. 3*B*). These cysts were apparently the remains of previously injured glands. No occlusion was found in the lumens of these glands which would produce obstruction and cause the formation of cysts. Sometimes a portion of the wall of a cyst was lined by either large cuboid epithelial cells, flat cells resembling those found normally in the basement membrane or hyperchromatic columnal epithelial cells (fig. 2*B*). Only a few mitotic figures were

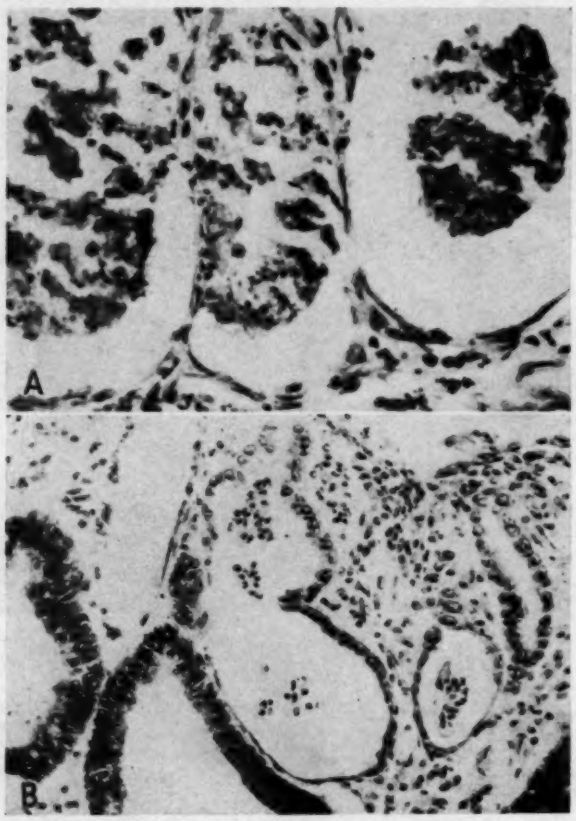

Fig. 2.—*A* (dog 47), necrotic, desquamated lining epithelial cells of glands in the mucosa; × 800. This dog was given an injection of 4 cc. of staphylococcus toxin directly in the colon and killed twenty-four hours later. *B* (dog 22), regenerating glandular epithelium; × 450. Note the variation in cell type. Goblet cells are apparently absent. There is some proliferation of fibrous tissue in the stroma of the mucosa. This dog was killed four days after receiving an injection of 6 cc. of staphylococcus toxin directly in the colon.

found in areas of mucosa showing regeneration. Goblet cells were not observed during the early stages of repair.

The colons examined on the seventh to the eighth day after the injection of toxin showed local areas of ulceration, and the mucosa around the ulcers was atrophic.

The colon from one dog examined at this time showed only focal areas of atrophic mucosa (fig. 1*C*).

The stroma in the mucosa of the colon showed a lesion comparable to the one that occurred in the glandular epithelium. When extensive injury occurred in the mucosa there was a proliferation of fibrous tissue with a decrease in the number of glands (fig. 3*C*).

During the first few days after the injection of the toxin the submucosa was usually edematous and infiltrated with few red blood cells and polymorphonuclear leukocytes. The blood vessels and lymphatics were often surrounded by cells of the latter type. Some of the endothelial cells were swollen, and others were

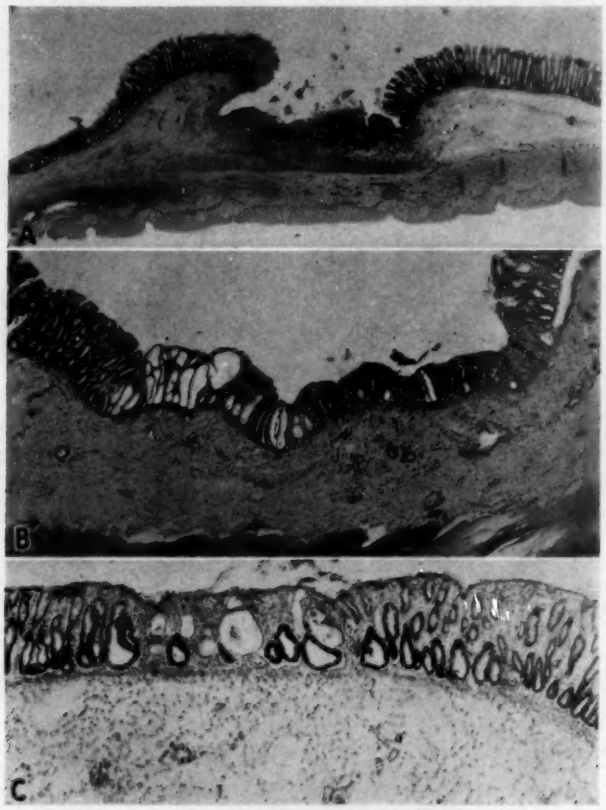

Fig. 3.—*A* (dog 44), ulceration of the mucosa and submucosa; × 14. Staphylococcus toxin was injected into the colon four days previously. *B* (dog 46), necrosis of the mucosa of colon; × 35. The cystlike spaces to the left are the remains of necrotic glands. This dog was killed three days after receiving 4 cc. of staphylococcus toxin directly in the colon. *C* (dog 22), atrophy of the mucosa with a decrease in the number of glands and an increase in the stroma; × 55. Note the collection of leukocytes about the blood vessels in the submucosa. This dog received an injection of 6 cc. of staphylococcus toxin locally in the colon four days before it was killed.

degenerated. The walls of some of the larger arteries were infiltrated with leukocytes. Fresh thrombi either partly or completely filled the lumens of a few of these vessels.

Foci of necrosis and fibrosis were the usual lesions found in the muscular layer of the colon. Some of the blood vessels in this area of the colon were surrounded by an inflammatory reaction, which apparently was associated with a similar inflammatory reaction in the serosa.

The serosa in almost every dog showed some evidence of an inflammatory process. The lesions varied from an acute inflammatory reaction to granulation tissue with organization.

Dog 25 was moribund thirty hours after the injection of toxin. He was killed at this time. The abdominal cavity was filled with a thin brown fluid, which apparently contained fecal material. The colon was gangrenous except for 2 to 3 cm. adjacent to the cecum.

One of the two dogs in the control group was put to death on the fourth postoperative day. The colon was normal. The second dog was put to death on the eighth postoperative day. This dog's colon was also normal except for a few petechiae in the serosa.

COMMENT

This study shows that the lesion produced in the dog's colon by local injection of the toxin of Staphylococcus aureus is similar to the lesions previously described in the rabbit's and the dog's colon following intravenous injection of staphylococcus toxin.[6] This pathologic process is also similar to one found in the gastro-intestinal tract of each of two children who died with staphylococcic toxemia.[3]

It is suggested that staphylococcus toxin produced injury to the mucosa of the colon through its action on the individual cells. Epithelial cells apparently are more easily injured by this toxin than the stromal cells. The union of toxin with cell apparently occurs very quickly and is irreversible. The action of staphylococcus toxin on tissues of different types appears to be similar. Little is known about the chemical reaction between tissue cells and toxins. Staphylococcus toxin, however, produces a change in the cell that resembles coagulation of the cytoplasm.

The necrosis of the skin following intracutaneous injection of toxin is similar to the necrosis of the colon which follows local injection. In view of the similarity in the types of necrosis produced by staphylococcic toxin in the different tissues, it seems unlikely that there should be two fractions in the toxin, one a "skin-necrotizing" and the other a "gastro-intestinal" toxin.[7]

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6. Rigdon.[1] Rigdon and Leff.[3]

7. von Lingelsheim, W.: Aetiologie und Therapie der Staphylokokkeninfectionen, Berlin, Urban & Schwarzenberg, 1900. Dolman, C. E.: J. Infect. Dis. **55**:172, 1934.

The regeneration of the mucosa observed in this study was similar to that previously described by Cornil and Ranvier.[8] Sach [9] described "cysts" in the gastric mucosa of dogs which were poisoned by tartar emetics. These "cysts" resembled the cystlike spaces observed in areas of the mucosa of the colon following local injection of staphylococcus toxin.

SUMMARY

Staphylococcus toxin when injected locally into the mucosa and submucosa of the dog's colon produces degeneration of the glandular epithelium and stroma. The ulcers which follow the desquamation of this necrotic mucosa apparently heal in from eight to fourteen days.

The regeneration of tissue following local injection of staphylococcus toxin is similar to the regeneration of the mucosa following other types of injury.

8. Cornil, V., and Ranvier, L.: Manuel d'histologie pathologique, ed. 3, Paris, F. Alcan, 1912, vol. 4, chap. 1, p. 152.

9. Sach, A.: Arch. f. exper. Path. u. Pharmakol. **22**:155, 1887.

CHANGES IN THE LIVER OF THE CAT FOLLOWING LIGATION OF SINGLE HEPATIC DUCTS

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AND

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Few studies of the effects of ligation of single hepatic ducts in the cat have been reported, and no detailed descriptions of the early changes that occur in the liver of this animal under such circumstances are available. In view of the marked differences which various species exhibit on obstruction of the bile passages, this study was undertaken chiefly for the purpose of comparing the effects of obstruction of single lobes of the liver of the cat with those of total bile stasis as reported previously.[1]

MATERIAL AND METHODS

Seventeen adult cats weighing from 1.4 to 3.2 Kg. were employed in the study. They were maintained on a diet of fresh raw scrap meat and milk. Under light ether anesthesia, one or more ducts leading from the left lobes of the liver were doubly ligated and divided. In eight cases the ligatures were so placed that the gallbladder communicated with the obstructed portion of the duct system. In each instance the exact site of obstruction was determined at autopsy. After periods of stasis of from one day to approximately five months the animals were killed by bleeding under light ether anesthesia. Just before death estimations were made of the bromsulphalein retained thirty minutes after injection of 2 mg. per kilogram, of the bilirubin in the serum (van den Bergh) and of the urobilinogen in the urine. This series includes no case in which there was evidence of infection or of patency or reconstruction of the obstructed ducts. In every instance bile was present in the duodenum and could be expressed into it from the unobstructed portion of the duct system.

Tissues were fixed in a solution of formaldehyde (1:10) or of formaldehyde with Zenker's solution.[2] Some were subsequently frozen, cut and stained with

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Aided by the Jefferson Medical College Hospital Tumor Clinic and the Martin Research Fund.

From the Departments of Pathology and Medicine, Jefferson Medical College of Philadelphia, and the Laboratory of Biochemistry, Jefferson Medical College Hospital.

1. Stewart, H. L., and Lieber, M. M.: Arch. Path. **19**:34, 1935.

2. The formula is: potassium chromate, 2.5 Gm.; mercury bichloride, 5 Gm.; distilled water, 100 cc., and neutral solution of formaldehyde (40 per cent), 10 cc.

nile blue sulfate and scarlet red. The remainder were blocked in paraffin, cut and stained with hematoxylin and eosin. Frozen sections (from 8 to 25 microns thick) were examined under crossed Nicol prisms for doubly refractile material.

GROSS CHANGES

Clinically, the animals remained in good condition, ate well and, in contrast to those with total bile stasis, did not appear to be unusually susceptible to infection.

At the end of one day, no difference in appearance could be detected between obstructed and nonobstructed portions of the parenchyma of the liver. From the third to the sixteenth day, only slight changes were discernible, consisting chiefly in accentuation of lobular markings in the obstructed lobes, with congestion and occasionally lobular atrophy and increased consistency. In one case no gross difference could be detected after six days. Subsequently, the distinction between the two portions of the liver became more evident, the obstructed lobes being usually slightly firmer, the lobules smaller and the markings more prominent. In one instance (twenty days), the obstructed lobes were larger and paler, with rounded edges, owing apparently to edema. The obstructed lobes at times appeared somewhat smaller and the nonobstructed slightly larger than normal, but this difference in size was by no means striking. In the late stages of the experiment the surface of the obstructed lobes was at times finely granular, and in one case (fifty days) presented large nodular projections due to enormous dilatation of intrahepatic ducts.

The ducts leading from the nonobstructed lobes showed no change and contained thin pigmented bile. The obstructed extrahepatic ducts underwent progressive dilatation with thickening and fibrosis of their walls, these changes being less marked than in total stasis of equal duration. In only two cases (thirty-five and fifty days) did the dimensions of the extrahepatic ducts exceed four times their normal diameters. The obstructed ducts contained "white bile" in all but four cases (one, five, thirty-five and forty-five days), in the first two of which the bile was light brown and in the last two pale green. Flaky masses of leukocytes were present in the obstructed ducts in one instance (sixteen days). In the cases in which the gallbladder communicated with the obstructed portion of the duct system it was generally moderately distended and thickened and its mucous membrane hyperplastic. The bile in the gallbladder was thick, ropy and dark green except in one case (twenty days), in which it was thin and colorless. Although the intrahepatic ducts underwent progressive thickening and fibrosis, dilatation was slight as compared with that in total stasis of equal duration, except in one instance (fifty days).

MICROSCOPIC OBSERVATIONS

Nonobstructed Lobes.—The hepatic cells in the nonobstructed lobes showed no evidence of degeneration or regeneration. Throughout the course of the experiment, minute collections of inflammatory cells were scattered diffusely in the sinusoids. After the eighth day the small portal radicles were unusually prominent, and in some instances there appeared to be slight proliferation of the small bile ducts. In a few cases there was rather marked pigmentation of both hepatic and Kupffer cells.

Obstructed Lobes.—Pigmentation: The degree of pigmentation of the hepatic and Kupffer cells was variable, never striking and, in many instances, not materially different from that of the nonobstructed portion of the parenchyma.

Regressive Changes: Neither diffuse nor sporadic degenerative changes were as marked as in animals with total stasis of equal duration. In fact, with the exception of extensive vacuolation, particularly in the later stages, the hepatic

parenchyma was surprisingly free from regressive changes. Slight to moderate degeneration and necrosis were occasionally observed about the central vein or portal areas or both, but frequently no such lesions were observed. There was no demonstrable relationship between the extent of regressive changes and the duration of stasis. In one animal (six days) there were sporadic areas of hyaline necrosis about the portal radicles and in the subcapsular zone, one such area in the latter situation showing evidence of organization. Characteristic areas of focal midzonal necrosis were observed in three cases (five, twelve and forty days), resembling those described elsewhere[1] in cats with total bile stasis.

The hepatic cells bordering the portal radicles and to a lesser extent those in the remainder of the periphery of the lobules presented a rather distinctive appearance in all cases. These cells were smaller and flatter than normal, with deeply staining smooth homogeneous cytoplasm, which frequently stood out in sharp contrast to the extensively vacuolated hepatic cells in the interior of the lobules. Degenerative and necrotic changes were sometimes present in these cells, especially at the points at which the proliferating bile ducts penetrated the lobule, and it was often difficult to distinguish between hepatic cells and cells of bile ducts in this situation. These deeply staining hepatic cells were most prominent in the later periods of stasis.

From the third day on, a variable degree of lobular atrophy was observed, the diminution in size never exceeding a third. The atrophied lobules were sharply outlined, partly by the proliferated small portal radicles, but chiefly by the narrow cords of deeply staining hepatic cells described.

Alterations in the Bile Ducts and Connective Tissue: The changes in the bile ducts and associated connective tissue were essentially of the nature of those described in total stasis.[3] In the first sixteen days the smallest bile ducts showed relatively slight proliferation, which increased rather rapidly subsequently, although occasionally proliferation was only slight in the later stages of stasis, notably in one animal after forty-five days. These proliferated ducts were tortuous; frequently they were well lumenized, particularly near their points of origin, and their lining cells were at times flattened and at times irregular and hyperchromatic; frequently the lining cells contained mitotic figures. Although the degree of proliferation of connective tissue was generally of the same magnitude as that of the small bile ducts, this was not invariably the case. Disappearance of proliferated ducts through accumulation of hyaline secretion[1] was noted during the first twenty days but was not as prominent as in animals with total bile stasis.

As the period of stasis increased, the hepatic lobules gradually became more sharply outlined by (1) the deeply staining smooth liver cells described previously and (2) the proliferated small bile ducts and associated connective tissue. The latter extended outward from the portal areas in fine parallel strands, which were well hyalinized in the portal regions and more cellular distally. These strands, together with the proliferated bile ducts, approached similar projections from adjacent portal radicles until, in cases of prolonged stasis, many of lobules were completely surrounded. At the point of deepest penetration into the hepatic tissue it was often difficult to distinguish between fibroblasts, cells of bile ducts and the deeply staining smooth hepatic cells. Despite the fact that two, three and sometimes four projections of connective tissue extended perilobularly from the portal radicles, there was rarely any marked tendency of this tissue to invade the lobules (fig. 1). The centers of the portal radicles usually contained collections of small round cells, mononuclears and occasionally polymorphonuclear leukocytes, but these were seldom

3. Cantarow, A., and Stewart, H. L.: Am. J. Path. **11**:561, 1935. Stewart and Lieber.[1]

numerous in the perilobular projections of connective tissue and bile ducts. The distribution of these infiltrating cells was not uniform, some portal areas in obstructed lobes being at times practically free from them while others were extensively involved.

In the first few days of stasis the mucosa of the medium-sized and larger bile ducts underwent proliferation, the lining cells becoming irregular and hyperchromatic and frequently containing mitotic figures; the walls of the ducts became slightly fibrotic, and some of the papillary projections of mucous membrane were infiltrated by inflammatory cells. Small masses of bile pigment were occasionally

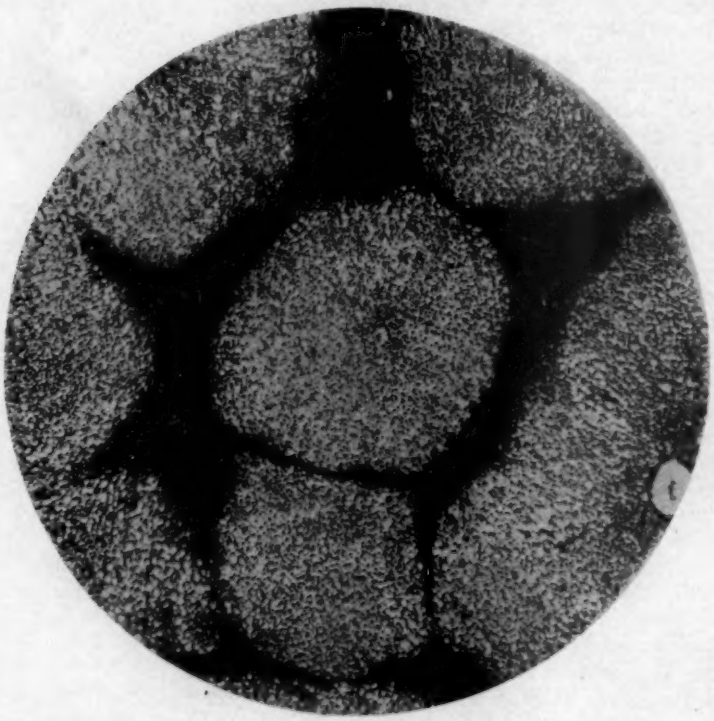

Fig. 1.—Section of liver from a cat with stasis for sixty-two days, showing the strictly perilobular fibrosis; magnification about × 50.

observed in the mucosal crypts of the medium-sized ducts. As the period of stasis increased the ducts became dilated and their walls thickened, and mucosal proliferation became more extensive, the papillary folds being occasionally lined by two or three layers of irregular, hyperchromatic epithelial cells, often observed in mitosis. The walls of the large and medium-sized bile ducts became progressively thickened with proliferating connective tissue, which had a rather distinctive loose fibrillary arrangement and many large pale vesicular fibroblastic nuclei scattered throughout. In the large ducts, many small blood and lymph vessels could be seen coursing in all directions within this loose connective tissue, lined by swollen endothelial cells and frequently surrounded by nodular collections of small round cells, mononuclears and polymorphonuclear leukocytes. Occasionally remnants of

glandular structures were observed in the walls of these ducts, the individual cells being compressed and necrotic and in various stages of disintegration. The original periportal connective tissue also underwent progressive proliferation, the thickened large and medium-sized ducts and the associated blood and lymph vessels being first surrounded by and later incorporated within a thick collar of dense hyalinized connective tissue, which stood out in sharp contrast to the cellular fibrillar connective tissue described. These changes were more prominent in the case of the large than in that of the medium-sized ducts. Eventually, the picture presented was that of enormously thickened bile ducts, in the outer, densely hyalinized walls of which were incorporated the compressed portal blood and lymph vessels.

The medium-sized ducts were gradually compressed by the proliferating connective tissue, their lumens becoming slitlike, with atrophic and degenerative changes in the lining epithelium. These ducts could be observed in various stages of occlusion and destruction up to complete replacement by large patches of solid fibrous tissue (fig. 2*A*). The larger bile ducts underwent gradual dilatation, which eventually became marked; the mucous membrane, at first hyperplastic with large papillary projections, later became stretched and was frequently lined in places by only a single layer of epithelial cells, interrupted by slight projections representing the vestiges of the previously hyperplastic papillae. The epithelial cells were chiefly columnar, but some were cuboid; they were generally well preserved, although some were compressed, and occasional cells were necrotic.

Vascular Changes: The sinusoids were diffusely infiltrated by a fine scattering of inflammatory cells, which were more numerous than in the nonobstructed lobes. This infiltration was often most prominent in the inner portions of the lobules, between the narrow cords of flattened hepatic cells. In the early stages of stasis the vascular structures in the portal areas were dilated and congested, but later they became compressed by the surrounding fibrous tissue, their lumens becoming slitlike. In one animal (sixty-two days) an organizing thrombus was present in a branch of the portal vein. No evidence of infarction of the liver was observed in any animal in this series.

Regeneration: Evidence of hepatic cell regeneration was observed throughout the entire experiment, consisting of hyperchromatism and binucleation, mitosis and the presence of flattened cells, particularly in the inner portion of the lobule. Mitotic figures were present in hepatic cells from the third to the fiftieth day, being most numerous between the twelfth and thirty-fifth days. They were observed in all portions of the lobule, in association with both the flattened cells in the interior and the deeply staining cells in the periphery and also in relation to areas of focal midzonal necrosis.

Lipid (Table): Stainable lipid was present in practically the same amounts in the hepatic cells of both obstructed and nonobstructed lobes throughout the course of the experiment. Stainable lipid was present in the Kupffer cells in appreciable quantity in only three cases (three, eight and forty days). The amount and distribution of doubly refractile material varied considerably but were essentially the same in obstructed and nonobstructed lobes in each instance. At twenty, thirty and thirty-five days, although obstructed and nonobstructed lobes contained equal quantities of stainable lipid and doubly refractile material in both hepatic and Kupffer cells, the obstructed portions were distinctly more vacuolated than the nonobstructed. The deeply staining smooth hepatic cells at the periphery of the lobules of the obstructed lobes seldom contained more than minimal quantities of lipid.

Zone of Transition Between Obstructed and Nonobstructed Lobes.—After the twentieth day, sections cut to include the junction of obstructed and nonobstructed parenchyma showed a sharp line of demarcation between the two. At this stage

EXPLANATION OF FIGURE 2

A, section of liver from a cat with stasis for fifty days, showing in the center a bile duct undergoing compression and mucosal atrophy and degeneration; magnification about × 100. The entire portal radicle, including the bile duct and the blood vessels, is incorporated within an extensive area of densely hyalinized connective tissue, which is infiltrated by inflammatory cells.

B, section of liver from a cat with stasis for sixty-two days, showing a sharp line of demarcation between the obstructed and the nonobstructed lobules of the same lobe; magnification about × 50. Note the atrophy and practically complete perilobular fibrosis on the left (obstructed) and the absence of atrophy and fibrosis on the right (nonobstructed).

of stasis, the proliferated small bile ducts and associated connective tissue and the deeply staining smooth peripheral hepatic cells contrasted sharply with the essentially normal characteristics of the parenchyma of the nonobstructed lobules. The transition from obstructed to nonobstructed parenchyma was so sharp that often single lobules were observed surrounded over one half of their circumference by proliferated portal radicles and the deeply staining hepatic cells, the corresponding half of the lobule being more vacuolated than the other, which presented an essentially normal appearance throughout (fig. 2*B*).

HEPATIC FUNCTION

Abnormal retention of bromsulphalein was not observed in a single instance at any period of stasis.

The concentration of bilirubin in the serum was apparently normal (no visible reaction with the van den Bergh reagent) in all except the following three cases,

Stainable Lipid and Doubly Refractile Material in Obstructed and Nonobstructed Lobes of the Liver

Days of Stasis	Stainable Lipid				Doubly Refractile Material			
	Nonobstructed		Obstructed		Nonobstructed		Obstructed	
	Hepatic Cells	Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells	Kupffer Cells
3	++++	++++	++++	++++	±	++++	+	++++
6	++	±	++	±	±	++	±	++
8	.....	.....	++++	+	.....	.....	0	++
9	++++	±	++++	±	0	±	0	±
12	+++	±	+++	±	0	±	0	±
16	++++	±	++++	±	++++	++++	++++	++++
20	++++	±	++++	±	±	++++	±	++++
30	++++	±	++++	±	±	++++	±	++++
35	++++	±	++++	±	++	++++	++	++++
40	++++	+	++++	++	±	+	±	+
45	++++	±	++++	±	+	++	+	+++
50	++++	±	++++	±	+	+	+	±
62	++++	±	++++	±	++	++++	++	++++

in each of which a positive direct van den Bergh reaction was obtained: stasis five days, serum bilirubin 0.29 mg.; stasis eight days, serum bilirubin 0.20 mg. stasis, twelve days, serum bilirubin 0.20 mg.

Reactions positive for urobilinogen (method of Wallace and Diamond) with dilutions of urine greater than 1:20 were obtained as follows: stasis five days, 1:40; stasis eight days, 1:50; stasis nine days, 1:50; stasis twelve days, 1:40; stasis sixteen days, 1:40. In each of these cases the gallbladder either communicated with the obstructed ducts or was obstructed independently by ligation of the cystic duct.

COMMENT

Previous studies indicate that different species react differently to ligation of single hepatic ducts as they do to ligation of the common bile duct. Nasse [4] in 1894 ligated the duct of the main liver mass in the rabbit and noted marked and progressive atrophy of the affected tissue

4. Nasse, D.: Arch. f. klin. Chir. **48**:885, 1894; Semaine méd. **14**:202, 1894.

(50 per cent shrinkage in six weeks) and to a corresponding degree hypertrophy of the nonobstructed portion of the liver. A few years later, Harley and Barratt [5] in similar experiments on cats found little if any gross atrophy or hypertrophy after from five to six months, practically the only superficial difference between obstructed and nonobstructed lobes being more marked accentuation of lobular markings in the former. Distinct atrophy of the affected lobes was noted after from twelve to fifteen months. The observations of Nasse on rabbits have been confirmed by Rous and Larimore [6] and Rous and McMaster,[7] who also found that these changes were much less pronounced in the dog and the cat. Our observations during stasis of relatively brief duration are in keeping with those mentioned in the case of the cat, although careful investigation often revealed early differences of slight degree between obstructed and nonobstructed portions of the liver; these consisted chiefly in slight accentuation of lobular markings, slight lobular atrophy and some increase in density in the obstructed lobes. Although there was little gross evidence of hypertrophy of nonobstructed lobes, these were at times slightly larger than the obstructed lobes and microscopically showed in some cases unusually prominent small portal radicles and slight proliferation of the small bile ducts.

There are certain points of interest in connection with the contents of the obstructed bile ducts. Harley and Barratt [5b] stated that in a cat examined after twelve months the bile was light brownish yellow, clear and somewhat viscid. In an earlier communication [5a] they stated that little difference was noted in the bile in the obstructed and nonobstructed ducts of cats with stasis of from four to five months' duration. According to Rous and McMaster,[7] whereas obstructed ducts draining entire lobes contain colorless bile with no bile salts, obstructed ducts from other lobes which are in part drained by nonobstructed ducts are filled with bile which is regularly yellow, containing bilirubin and cholates. This influence of a free duct on what should be a "white system" is attributed by them to an absence of absolute physiologic separation of the normal hepatic parenchyma and the region in stasis, although anatomically there is a sharp line of demarcation between the two. Rous and McMaster stated that

> the fact that the pigment in the affected white system is bilirubin and not biliverdin as in old stasis bile argues its recent production. The most reasonable explanation of its presence would seem to be a constant slight secretion of bile into the ducts as a result of a lowering of pressure in them consequent in turn upon a slight continual leakage from the white system over into the adjoining unobstructed

5. Harley, V., and Barratt, W.: (*a*) Brit. M. J. **2**:1743, 1898; (*b*) J. Path. & Bact. **7**:203, 1901.

6. Rous, P., and Larimore, L. D.: J. Exper. Med. **32**:249, 1920.

7. Rous, P., and McMaster, P. D.: J. Exper. Med. **34**:75, 1921.

region. How and when the leakage occurs cannot be said. In the monkey there is evidence for a considerable amount of it in the development regularly of a broad zone of transition between the liver tissue that is atrophic as the result of transmitted pressure from the obstructed ducts and the adjoining normal parenchyma.

Our observations in this connection are not in complete accord with those previously reported. Despite the fact that the portion of liver tissue in stasis almost invariably included a portion of a large lobe in which the remainder was drained by a free duct, the obstructed ducts contained "white bile" in all but four cases (one, five, thirty-five and forty-five days of stasis), in the first two of which the bile was light brown and in the last two pale green. This, together with the presence of a sharp line of intralobar demarcation between obstructed and non-obstructed hepatic tissue, suggests an absolute physiologic as well as anatomic separation of the two portions of the parenchyma in the cat.

Regressive changes in the hepatic cells of the obstructed lobes differed from those which occur in total bile stasis [1] chiefly as follows: 1. Degeneration and necrosis were much less extensive; (2) focal necroses developed at a later period of stasis and less frequently; (3) the hepatic cells at the periphery of the lobules underwent a rather distinctive change, their cytoplasm becoming smooth and staining deeply; (4) lobular atrophy progressed inward from the periphery of the lobules, the cells in the interior frequently appearing essentially normal. Previous reports contain no detailed observations regarding regressive changes in the early stages of unilobar stasis with the exception of the statement by Rous and Larimore [6] that in the rabbit biliary necroses are small and appear only during the first few days. All authors agree that the effects of sudden stasis of single lobes are less marked than are those of total bile stasis.

As has been noted by previous observers, proliferation of the small bile ducts and associated connective tissue in the affected lobes was distinctly less extensive than in total stasis. It is interesting to note the slight proliferation of small bile ducts in the nonobstructed lobes, but the significance of this observation is not apparent. Our findings coincide with those of Harley and Barratt [8b] and with those of Rous and Larimore [6] in more prolonged stasis in regard to the nature of the cirrhotic process, which differs from that of total stasis in its slow, orderly progress and the almost complete failure of the proliferating ducts and connective tissue to invade the lobule. Changes in the larger and medium-sized intrahepatic ducts were essentially the same as in total stasis, but their much more gradual progression afforded an opportunity to observe certain details that were obscured because of the rapidity of progress of these phenomena in total stasis. This was true particularly of the mechanism whereby the wall of the duct became

thickened with proliferating loose fibrous tissue and subsequently encircled by dense hyalinized connective tissue in which were incorporated the adjacent blood and lymph vessels, the latter being eventually markedly compressed and the ducts at times completely obliterated.

The gradual atrophy of the lobules from without inward and the almost completely unilobular nature of the cirrhotic process were noted and emphasized by Harley and Barratt [5b] in cats and by Rous and Larimore [6] in rabbits. The latter ascribed this progressive atrophy and orderly cirrhosis to the interaction of two opposing sets of factors, the one deleterious and the other beneficial and ameliorating. Among the first are the immediate consequences of increased intraductal pressure, the dilatation and rupture of the finer channels, with resulting parenchymal necrosis and interlobular and pericholangitic fibrosis, and disturbance of the blood supply by the pressure of dilating ducts and contracting fibrous tissue. Chief among the limiting factors is the circumstance that the unobstructed portions of the liver, by preventing excessive retention of biliary constituents in the organism, tend to limit the amount of resecretion of these substances into the area of stasis, this resecretion being possibly further diminished by the diverted portal blood supply. Rous and Larimore stated also that as the lobular atrophy progresses the pressure of bile falls and hence rupture of the ducts and extravasation of bile eventually cease, the functional demands on the obstructed lobules being further reduced by subsequent increased activity of the hypertrophied nonobstructed portions of the parenchyma. "The predominantly monolobular cirrhosis which follows the occlusion of a single duct, and the purely monolobular lesion that develops when the corresponding portal branch is also tied, are referable not to rupture of the ducts, which is negligible, but to continued passage through their walls of the irritant bile" (Rous and Larimore [6]). This interpretation seems to us to be logical on the basis of our present observations.

Previous authors have made little mention of evidences of hepatic cell regeneration in the obstructed lobes. It is interesting to note that Harley and Barratt [5a] in 1898 recorded the observation of narrowed columns of hepatic cells which, in the light of recent knowledge, may probably be regarded as a manifestation of a reparative process. These observers also occasionally noted new hepatic cells in the area of stasis. In the present series, the observation of hyperchromatism, binucleation, mitosis and flattening of the cells in the interior of the lobule leaves no doubt as to the activity of hepatic cell regeneration throughout the duration of the experiment. In total stasis in the cat [1] mitotic figures were most numerous in the first fifteen days and were rarely seen in association with cells of flattened type in the inner portion of the lobule. It may perhaps be of significance that in the present study mitotic figures

were most numerous in the obstructed lobes between the twelfth and thirty-fifth days and were also noted in association with cells of the flattened type. This persistence of evidences of regeneration and repair in later periods of stasis of single lobes is in keeping with the more gradual progress of all other changes that develop under these conditions.

Harley and Barratt [5b] recorded the presence, in one case, of much more fat in the normal than in the obstructed portions of the liver. They stated that in the latter the atrophied lobules showed here and there cells loaded with fat lying beside columns of hepatic cells. Study of their illustrations reveals that these cells are probably Kupffer cells. Whereas in a previous study of the effects of total bile stasis in cats [8] we found that stainable lipid practically disappeared from both hepatic and Kupffer cells after the seventh day, in the present experiments there was essentially no difference in the amount of this substance in obstructed and nonobstructed lobes after periods of stasis up to sixty-two days. However, it is of interest to note that the deeply staining cells in the periphery of the affected lobules usually contained little or no stainable lipid. We believe it to be of significance that, as in total stasis, there was no constant relationship between the presence and extent of regressive changes and the distribution and quantity of fat in the liver, suggesting that the latter are probably dependent on functional phenomena rather than on degenerative changes.

Previous investigators have stated that jaundice does not occur following ligation of single ducts, but no data are available regarding possible alterations in the serum bilirubin, such statements having been based apparently on the absence of visible pigmentation and bilirubinuria. Concentrations of from 0.2 to 0.29 mg. of bilirubin per hundred cubic centimeters of serum in three cases in the present series indicate that distinct hyperbilirubinemia may occur under such circumstances. Furthermore, although abnormal retention of bromsulphalein did not occur in any case in this series, impairment of hepatic function was suggested in five instances by urobilinuria to a degree apparently excessive for the cat. The data at hand are insufficient to allow a definite statement to be made in this connection, but in our experience the detection of urobilinogen in dilutions of urine greater than 1:20 is unusual for the normal cat, as for man. In each of the cases in which bilirubinemia or urobilinuria was excessive the gallbladder either communicated with the obstructed ducts or was obstructed independently by ligation of the cystic duct. The significance of this fact is not readily apparent.

8. Cantarow, A.; Stewart, H. L., and Lieber, M. M.: Arch. Path. **20**:535, 1935.

SUMMARY

The changes in obstructed portions of the liver following ligation of single hepatic ducts are, with few exceptions, essentially similar to those which result from ligation of the common bile duct. All are, however, much less marked and less rapidly progressive. The chief additional points of difference are as follows: (1) The cirrhosis is almost purely monolobular; (2) focal necroses occur less frequently than in total stasis and develop at a later period; (3) the hepatic cells at the periphery of the lobules undergo a rather distinctive change, their cytoplasm becoming smooth and deep staining and containing little or no stainable lipid even when the remainder of the lobule shows large quantities; (4) lobular atrophy progresses inward from the periphery; (5) repair and regeneration of hepatic cells occur throughout periods of stasis up to sixty-two days; mitotic figures are most numerous between the twelfth and thirty-fifth days; (6) there is no essential difference in the amounts of stainable lipid in obstructed and nonobstructed lobes with periods of stasis up to sixty-two days; (7) demonstrable impairment of hepatic function may occur occasionally following ligation of single hepatic ducts.

HEMOBLASTIC LEUKEMIA

STUDY OF A CASE

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The difficulty of definite clinical diagnosis in this case of leukemia gives special point to an effort to interpret the blood picture in the light of data from a histologic study of various tissues secured at autopsy. These tissues included lymph nodes, spleen, femoral and vertebral marrow, liver, thymus, kidney and intestines. With the exception of the intestines, these tissues showed extensive lymphoid hyperplasia.

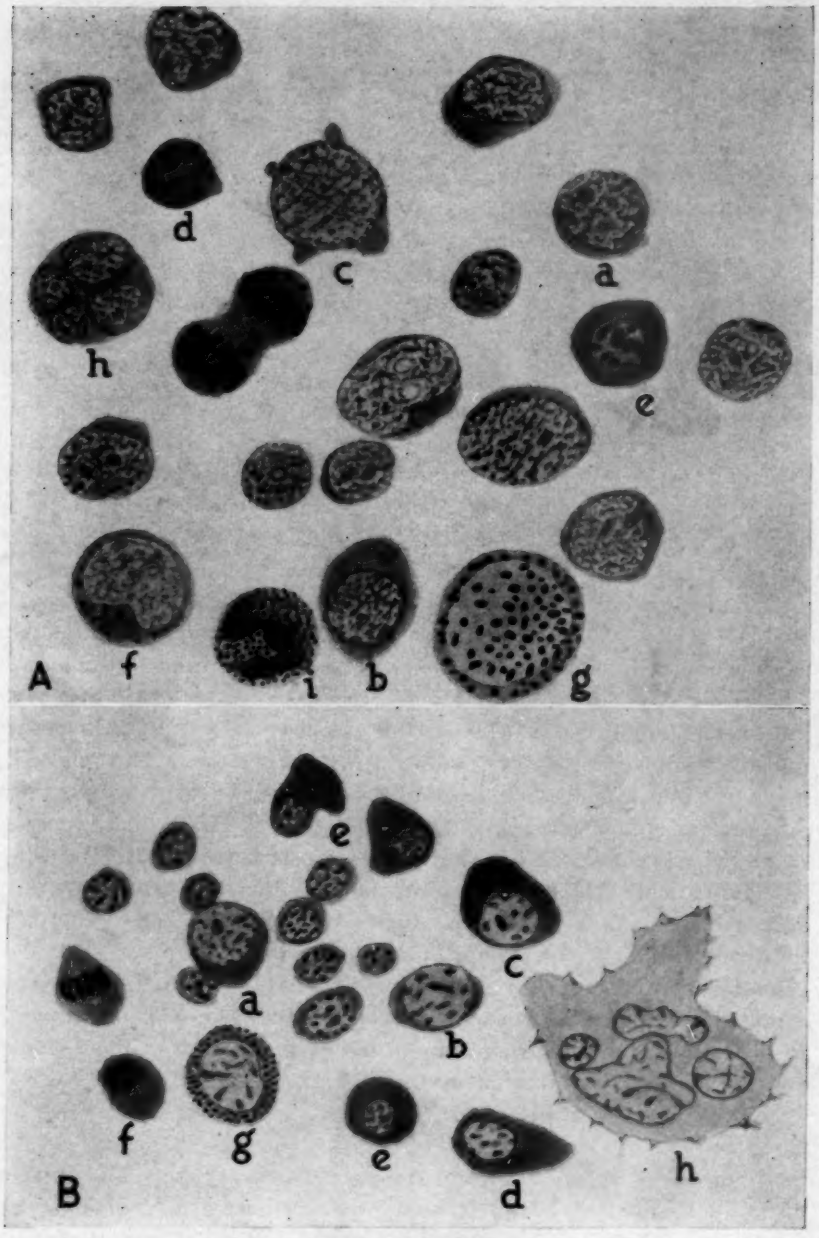
Death occurred on the eighth day after admission to the hospital. Red cell counts were made on the second and fourth days after admission; white cell counts, on the second, fourth, fifth and seventh days. The red cell count dropped from 2,150,000 to 2,080,000; the white cell count, from 640,000 to 450,000. The differential percentages remained approximately the same: polymophonuclear neutrophils, 2; polymorphonuclear eosinophils, 0.5; myelocytes, 1; large lymphocytes, 3.5; smaller lymphocytes, 93. The hemoglobin on the second day registered 30 per cent (Sahli). The lymphoid cells were oxydase negative.

The patient, acutely ill on admission, was a white girl 18 years of age. Unfortunately no earlier studies of the blood had been made. However, a fairly complete clinical history was secured. The data essential for the purposes of the present study concern the following symptoms: headache and malaise for two months previous to admission to the hospital, swelling of glands in the neck and pain in the abdomen and the long bones for three weeks.

BLOOD

Figure 1 *A* shows a group of selected cells from a blood smear after application of Wright's stain, the various shades of blue and lilac being indicated by varying shades of gray. Small lymphocyte-like cells predominate. The cell designated by *a* illustrates the average condition: It has a relatively large nucleus with a lilac tint and a narrow envelop of light blue cytoplasm; the nuclear reticulum is close-meshed and delicate, and supports one or several nucleoli; the cytoplasm may contain a few small vacuoles, but azurophilic granules do not occur. The figure shows larger and smaller but essentially identical cells of this variety. Minor variations concern the number and staining inten-

From the University of Virginia.



EXPLANATION OF FIGURE 1

A, selected cells from a blood smear: (*a*) lymphoid cell (lymphoid hemoblast), the variety most abundant in the smears; (*b*) rare variety with relatively large amount of cytoplasm; (*c*) typical hemocytoblast; (*d*) "definitive" lymphocyte (rare); (*e*) transition from erythroblast to normoblast (rare); (*f*) myelocyte; (*g*) basophil leukocyte (rare); (*h*) lymphoid hemoblast with lobular nucleus; (*i*) polymorphonuclear neutrophil leukocyte. Wright stain. Magnification, × 1,200.

B, selected cells from a section of femoral marrow; the small lymphocyte is the predominating type: (*a*) hemocytoblast; (*b*) pro-erythroblast ("megaloblast"); (*c*) and (*d*) erythroblasts; (*e*) and (*f*) normoblasts; (*g*) myelocyte; (*h*) megakaryocyte. Helly fixation; eosin-azure stain. Magnification, × 1,200.

sity of the nucleoli, the relative amount of cytoplasm, the shape of the nucleus and the staining intensity of the cytoplasm. Nucleoli may be lacking, or the nucleus may contain one or several; these nucleoli may appear as light or dark, oval or spherical bodies, depending on their content of chromatin. Certain cells (*b*) may have a relatively wide envelop of cytoplasm, and the nucleus may be indented, reniform or lobulated. The larger of these lymphoid cells (*c*) have the cytologic characteristics of typical hemocytoblasts. The smaller lymphoid cells have a very similar and in many instances an identical cytologic character. A series closely graded as regards size can be arranged from the smallest to the largest of these lymphoid cells. Moreover, the lymphoid cells of each size exhibit the set of nuclear and nucleolar variations listed. An occasional cell of the larger variety may be seen in mitosis. These variations in size apparently represent only different stages of growth of the same stem cell (hemoblast); and the morphologic variations of the nucleus seem dependent in general on a time relation with reference to mitosis.

The erythrocytes, neutrophils (*i*) and eosinophils of this blood appear normal. Typical, so-called mature lymphocytes (*d*) are extremely rare. Occasional erythroblasts and normoblasts (*e*) are present. The smears contain also an occasional basophil (*g*) and immature granulocytes (*f*) at various stages of differentiation. In each of these promyelocytes (*f*) the nucleus closely resembles that of the larger lymphoid cell (hemoblast), and the fact that a series of transitional stages may be arranged between the two suggests a genetic relationship.

The evidence, then, from a study of the blood smears alone is equivocal as regards the specific type of leukemia. The predominating cell is a small lymphocyte-like cell. Similar cells of intermediate and large size are very numerous. The large lymphocyte-like cell resembles the typical hemocytoblast. The smaller cells are daughter cells of the large hemocytoblasts. Accordingly, the predominating cells are hemocytoblasts (hemoblasts). Since typical small lymphocytes are almost entirely absent, it seems clear that the lymph nodes and spleen are involved. Since myelocytes, erythroblasts and normoblasts occur, it seems equally clear that the bone marrow is involved. If one discriminates between lymphoblasts and myeloblasts, this is a case of either lymphoblastic or myeloblastic leukemia, depending on which tissue may be involved to a relatively greater degree; or if both are approximately equally involved, the better designation would be "mixed leukemia." If one considers lymphoblasts and myeloblasts identical cells located in lymph nodes and marrow, respectively, the case is one of hemoblastic leukemia. The latter term seems to fit the case best.

MARROW

Figure 2 *B* shows a collection of selected cells from a section of marrow from near the middle of the femur. The collection is representative of the average area. The marrow from a vertebra had essentially the same structure. The marrow had the general appearance of fairly compact lymphoid tissue. There were no lymphoid nodules. Megakaryocytes were very rare. Venous sinuses were not generally discernible. There were no larger collections of maturing erythrocytes and granulocytes such as characterize normal active marrow. Only very occasional and only very small groups of erythroblasts and myelocytes could be found. The maturing erythrocytes and granulocytes were widely scattered among the predominating lymphoid cells. Fat cells occurred, but they were relatively rare and widely scattered.

The commonest cell was one resembling a small lymphocyte, with a relatively large nucleus; the chromatin occurred in irregular blocks; the cytoplasm had a faintly blue color after eosin-azure staining. This small cell represented the first of a series of transitional stages ending in a cell of relatively large size with the cytologic features of the typical hemocytoblast (*a*), the "myeloblast" of marrow. This large cell functioned as a stem cell for the granulocytes (*g*) and could also be placed at the beginning of a series representing transitional stages, including the megaloblastic, erythroblastic and normoblastic phases of erythrocytogenesis (*b* to *f*). The large and medium-sized lymphoid cells proliferated extensively. The resulting daughter cells resembled typical small lymphocytes, the predominating cells of the marrow. These small lymphocytes hypertrophied and became typical hemocytoblasts, ancestors of both erythrocytes and granulocytes. The lymphoid cells of this marrow, then, were hemoblasts, the common ancestors for all varieties of blood cells. All of the cells identified in the blood smears (fig. 1 *A*) were represented in the marrow, and with the evidence from blood and marrow the clinical condition might be designated as one of myeloid or myeloblastic leukemia.

LYMPH NODES

The condition of the lymph nodes was surprising; it was practically identical with that of the femoral marrow. The lymph nodes had a uniformly compact structure; there was no demarcation between the cortex and the medulla; nodules did not occur. The peripheral sinus was narrow, and the fatty capsule was infiltrated with lymphocytes. Figure 2*A* illustrates a typical area of a mesenteric lymph node. The predominating cell was the small lymphocyte. Abundant transitional stages related it to the typical hemocytoblast, the "lymphoblast" (*a*) of

lymphoid organs. Many of the larger and medium-sized lymphocytes were in mitosis. The same relationship obtained here between small lymphocytes and hemocytoblasts as in the marrow; that is, hemocyto-

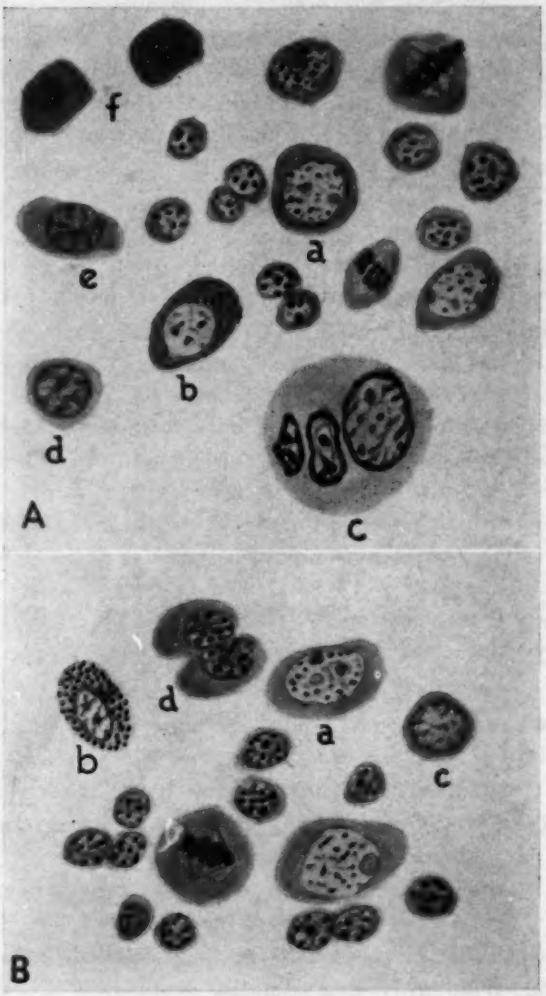


Fig. 2.—*A*, selected cells from a section of mesenteric lymph node; small lymphocytes predominate: (*a*) hemocytoblast (lymphoblast); (*b*) myelocyte; (*c*) megakaryocyte; (*d*) and (*e*) erythroblasts; (*f*) normoblasts. Zenker-formaldehyde fixation; eosin-azure stain. Magnification, × 1,200.

B, selected cells from a section of spleen; small lymphocytes predominate: (*a*) hemocytoblast; (*b*) myelocyte; (*c*) cell with the appearance of a proerythroblast; (*d*) cells with the appearance of erythroblasts (similar cells but with a juxtanuclear light oval area suggest plasma cells). Helly fixation; eosin-azure stain. Magnification, × 1,200.

blasts represented hypertrophied small lymphocytes; small lymphocytes were daughter cells of hemocytoblasts; medium-sized lymphoid cells were growth stages of this same series. The larger lymphocytes served, as indicated by abundant transitional stages, as stem cells for normoblasts (*d*), granuloblasts (*b*) and megakaryocytes (*c*). As was the case in the marrow, the lymph node tissue contained maturing erythrocytes and granulocytes, generally widely scattered, occasionally in groups of from 4 to 10 cells. Occasional basophils occurred; plasma cells were apparently lacking, and macrophages were rare.

The lymph node tissue was obviously in a condition of myeloid metaplasia. The marrow had the appearance of lymphoid metaplasia. The important fact, however, concerns the essential identity of the tissues from the lymph nodes and marrow. Both tissues were erythrocytopoietic and granulocytopoietic. The ancestral cells were the same in both, namely, hemocytoblasts. The differential designation of "lymphoblast" and "myeloblast" does not change their identical developmental potency. The smallest cells in the sections were typical small lymphocytes; in the blood smear they appeared identical with the larger hemocytoblasts. Accordingly, all of the lymphocytes may be considered potential hemoblasts. The evidence from a study of the lymph nodes, then, leads to the diagnosis of mixed leukemia, in the sense that the cells of the blood had origin both from lymph nodes and from marrow and that erythrocytogenic and granulocytogenic cells occurred in adjacent areas both in the lymph nodes and in the marrow.

SPLEEN

The spleen was definitely enlarged, weighing 660 Gm. and measuring 20 by 11 by 5.5 cm. But contrary to expectations, especially in view of the metaplastic condition of the lymph nodes, it gave only meager and uncertain evidence of erythrocytogenesis and very little evidence of granulocytogenesis. There were a few normoblasts and erythroblasts and only occasional basophils, young neutrophils and eosinophils. Macrophages were very rare. The splenic tissue had a compact uniform structure except for long columns of denser collections of small lymphocytes surrounding some of the larger arteries. These collections included larger hemocytoblasts (lymphoblasts) but nothing in the nature of definite germinal centers. Typical splenic nodules were lacking. The sinuses were well filled with lymphocytes, many in mitosis. The splenic enlargement resulted from lymphoid hyperplasia; very many of the medium-sized and some of the large lymphoid cells were in mitosis. The predominating cell here again was the typical small lymphocyte. Scattered among these were large

cells with the features of typical hemocytoblasts (fig. 2*B a*). The cytoplasm of these cells took a purple tinge after eosin-azure staining. Cells like that represented at *b* were fairly numerous. Their cytoplasm stained purple like that of the hemocytoblasts, but their nuclei resembled those of the small lymphocytes. They had some resemblance to plasma cells except that the juxtanuclear light crescent of typical plasma cells was lacking (fig. 2*B b*). They apparently represented daughter cells of the large hemocytoblasts and mother cells of the smallest lymphocytes. The condition of the spleen, then, resembled that of the lymph nodes and bone marrow, except that the spleen showed no erythrocytogenic activity and only slight granulocytopoietic activity.

THYMUS

The thymus had a uniform lymphoid structure with no demarcation evident between the cortex and the medulla of the lobules. The few persistent corpuscles of Hassall were small and atrophic and gave practically the only clue for the identification of the sections as thymus. The stroma and blood vessels were well filled with small lymphocytes. There were very few large cells. The lymphoid cells did not show mitotic figures. The thymus evidently had undergone lymphoid hyperplasia but was inactive at the time of death. There was no indication of either erythrocytogenesis or granulocytogenesis.

LIVER

The liver was characterized by periportal lymphocytic infiltration. Mingled among the predominating small lymphocytes were a few hemocytoblasts. There was only very slight proliferation, and evidence of differentiation into erythrocytes or granulocytes was lacking.

KIDNEY

As regards the kidney, there was considerable lymphocytic invasion of the fatty connective tissue of the hilus. Small lymphocytes predominated; hemocytoblasts were numerous. Numerous lymphocytes and a few hemocytoblasts were in mitosis. This area contained also a few megakaryocytes, and there were numerous transitional stages between hemocytoblasts and normoblasts and myelocytes, respectively. In short, this tissue was essentially identical with that of lymph nodes and marrow.

COMMENT

The central fact in this case concerns the essential identity in histology of the lymph nodes and marrow. Erythrocytogenesis and granulocytogenesis occurred in both tissues. This case clinically and

histologically is very similar to that described by Fineman [1] as microlymphoidocytic leukemia. Fineman's patient was a white woman of 17 years who had been well until about two months before admission to the hospital. She had enlarged lymph nodes on admission, and her blood picture included: hemoglobin, 29 per cent (Dare); red cells, 1,900,000; leukocytes, 99,000. During her stay in the hospital she was given blood transfusions and treatment with roentgen rays. During the two weeks preceding death the leukocytes rose from 17,000 to 646,000, lymphoid cells predominating. Fineman designated the predominating lymphoid cells "atypical cells" and stated that they were indistinguishable from typical myeloblasts. They had the cytologic features of typical small hemocytoblasts (lymphoid hemoblasts), and were identical with the cells designated "lymphoidocytes" by Pappenheim [2] and "micromyeloblasts" by Naegeli [3] and Schridde.[4] The only tissue available for study in this case was an axillary lymph node, removed at biopsy. There was no sign of atrophy, both nodules and cords showing many mitotic figures. Atypical cells (microlymphoidocytes) constituted the majority of the cells in the parenchyma and sinuses and were present even in the germinal centers. Fineman concluded that in all probability the majority of the myeloblasts of the blood came from the lymphoid organs, including the germinal centers of the cortical nodules, and that the evidence favored the unitarian interpretation of blood production.

Citron's [5] case also appears to have had points of close similarity. However, the bone marrow was said to be normal. The blood contained large numbers of "myeloblasts" ("lymphoidocytes"), identical with the cells of the splenic pulp and the interfollicular tissue of the lymph nodes and with those of the nodules. Since there was no evidence of nodular atrophy or of myeloid metaplasia of the interfollicular tissue, he concluded that the "myeloblasts" which could be seen entering the circulation from the hyperplastic lymph nodules were autocellular derivatives of nodular lymphocytes.

Logefeil [6] described a case of "mixed leukemia" which resembled the case of the present study in certain respects. Bone marrow was not available for study. The conditions in the lymph nodes, spleen, liver and kidney were similar to those described as regards especially

1. Fineman, S.: Arch. Int. Med. **29**:168, 1922.
2. Pappenheim, A.: Wien. klin. Wchnschr. **25**:163, 1912.
3. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Leipzig, Veit & Co., 1919.
4. Schridde, H.: Centralbl. f. allg. Path. u. path. Anat. **20**:433, 1909.
5. Citron, J.: Folia haemat. **20**:1, 1915.
6. Logefeil, R. C.: Arch. Int. Med. **33**:659, 1924.

the diffuse association of lymphoid and myeloid tissues in the lymph nodes. The present case differs markedly, however, in that the lymph nodes and spleen showed no atrophic areas and the lymph nodes gave evidence also of erythroid metaplasia, conditions very similar to those previously described in a case of acute leukemia (Jordan[7]) in which the marrow also showed extensive lymphoid metaplasia.

The present case resembles more nearly that reported by Hirschfeld,[8] in which there also occurred lymphadenoid metaplasia of the bone marrow and myeloid metaplasia of the lymph nodes and splenic pulp with a blood picture of lymphocytic leukemia. Hirschfeld concluded that the lymphatic leukemia was primary, and he was inclined to regard the lymphoid hemoblasts as ancestral cells of the myelocytes and possibly also of the nucleated red cells of the lymph nodes. He designated his case as one of mixed leukemia.

Since the lymph nodes and bone marrow had an essentially identical histology in the case of the present study, both containing a predominance of lymphoid cells with a mingling of maturing granulocytes and erythrocytes, the question arises regarding the primary source of the ancestral cells, whether lymph nodes, spleen or bone marrow. Leaving aside the doubtful possibility that the spleen may have been the primary source of the ancestral hemoblasts, the question narrows itself to the two alternatives of a marrow or a lymph node primary origin. The spleen at death was active only in the proliferation of lymphoid cells. If specific ancestral myeloid or erythroid cells were being transported in the blood stream from the marrow, they should have been present in various stages of differentiation in the splenic pulp as well as in the lymph nodes.

Assuming that the marrow was the exclusive source of the myeloid ancestors (granuloid and erythroid), the developing erythrocytes and granulocytes of the lymph nodes have to be interpreted in terms of transplanted marrow cells which found the conditions in the lymph nodes favorable for their differentiation into granulocytes and erythrocytes. Similarly, if one assumes that the lymph nodes (and spleen) were the exclusive source of the lymphoid cells, the abundant lymphoid cells of the marrow require the interpretation of metastases to bone marrow. Since the circulating blood contained all varieties of ancestral cells and transitional stages to definitive polymorphonuclear granulocytes, erythrocytes and alleged specific definitive lymphocytes, the possibilities were present for a transfer of ancestral myeloid cells from marrow to lymph nodes and for a similar transfer of lymphoid cells from lymph nodes and spleen to marrow. However, there is abundant

7. Jordan, H. E.: Arch. Path. **18**:1, 1934.
8. Hirschfeld, H.: Folia haemat. **3**:332, 1906.

evidence that lymphocytes occur normally in the bone marrow of man (Dominici,[9] Hedinger,[10] Oehme,[11] Askanazy,[12] Fischer[13] and Jordan[14]). And in the case of birds, which lack lymph nodes but contain numerous lymphoid nodules in the marrow, particularly in that of the femur and tibia, the evidence is especially clear that genuine small lymphocytes represent daughter cells of typical lymphoblasts (hemocytoblasts) and retain developmental potencies which may find expression either in erythrocytogenesis or in granulocytogenesis. The ancestral small lymphoid cells migrate into the venous sinuses of the marrow, where after a period of growth they mature into erythrocytes. Identical lymphoid cells in the intervascular stromal areas differentiate into granulocytes. In birds lymphoid tissue is a primary constituent of myeloid tissue. The two tissues are fundamentally and potentially one and the same. This conclusion is fully supported by the phylogenetic data concerning blood-forming tissue.

In cyclostomes the hemopoietic tissue is a disperse lymphoid spleen located in the submucosa of the intestine. Here are formed both erythrocytes and granulocytes. In lung-fish, also, the spleen is the principal hemopoietic organ; the more or less diffuse spleen is located within the wall of the stomach, and the submucosa of the intestine serves as an accessory source of granulocytes. In certain teleost fishes the extraenteral aggregate spleen is the chief hemopoietic organ; in other teleosts the intertubular stroma of the kidney serves as a considerable accessory source. In elasmobranchs erythrocytopoiesis is restricted to the spleen; granulocytopoiesis, largely to the stroma of the ovaries and testes. In most salamanders the red cell ancestors originate in the spleen; granulocytes are formed in the subcapsular area of the liver. In a few species of salamanders granulocytopoiesis is restricted to the kidney. Only in the anura (frogs and toads) does the bone marrow become active in the production of erythrocytes, and then only for brief periods following metamorphosis and hibernation (Jordan[15]).

In reptiles and birds the bone marrow becomes the chief seat of hemopoiesis. Only in mammals does the original multiple function of the spleen and its aggregation of three apparently different tissues become apportioned among lymph nodes, residual spleen and bone marrow. Throughout the vertebrate phylum a reticular stroma constitutes

9. Dominici, H.: Sang et moelle osseuse, in Cornil, A. V., and Ranvier, L.: D'histologie pathologique, ed. 3, Paris, Félix Alcan, 1902, vol. 2, p. 581
10. Hedinger, E.: Frankfurt. Ztschr. f. Path. **1**:527, 1907.
11. Oehme, C.: München. med. Wchnschr. **56**:446, 1909.
12. Askanazy, M.: Virchows Arch. f. path. Anat. **220**:257, 1915.
13. von Fischer, O.: Frankfurt. Ztschr. f. Path. **20**:347, 1917.
14. Jordan, H. E.: Am. J. Anat. **57**:1, 1935; **59**:249, 1936.
15. Jordan, H. E.: Quart. Rev. Biol. **8**:58, 1933.

the mother hemopoietic tissue. This reticular stroma appears identical in the spleen, lymph nodes and marrow of mammals. Cells separate from the syncytial stroma, essentially mesenchyme, and become hemocytoblasts. Identical cells, called "lymphoblasts" in lymph nodes and spleen, "myeloblasts" in bone marrow, represent in these separate tissues hemocytoblasts with developmental pluripotencies, proliferating as small lymphocytes in lymph nodes and spleen, and differentiating into erythrocytes or granulocytes in bone marrow, depending on their location in venous sinuses or in intervascular stroma.

In the light of these facts from comparative studies of homogenesis the identical histology of the lymph nodes and bone marrow of this case of leukosis seems to permit interpretation of the case as one of hemoblastic leukemia. The lymphoid tissue of both nodes and marrow represents identical fundamental hemopoietic tissue. The source of the hemoblasts in each tissue is chiefly local. Both tissues contribute to the enormous content of lymphoid cells in the blood, and there must necessarily be contributions in both directions between the lymph nodes and the marrow. The intense proliferative activity in the lymph nodes, marrow and spleen may be interpreted as indicating an effort to supply ancestral cells to combat the anemia and the agranulocytosis. The missing factor for normal hemogenesis seems to be one to support maturation of ancestral cells. The lymphadenosis and secondary anemia seem to be fundamentally the result of an accumulation of ancestral and unripe blood cells rather than of an abnormally rapid production of lymphocytes. The lymphoid cells are properly regarded as hemoblasts rather than lymphoblasts, and the typical small lymphocytes seen in the sections as ancestors of erythrocytes and granulocytes, which under certain morbid conditions are unable to mature and thus accumulate to produce leukemia.

HISTOLOGIC STUDY OF THE AMELOBLASTOMA

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The ameloblastoma structurally suggests an overgrowth of elements of the enamel organ. It is chiefly for this reason that a thorough understanding of the histology and embryology of the teeth is essential to an adequate interpretation of the etiology, occurrence and structure of this tumor. In this paper it is hoped that sufficient emphasis will be given to the basic dental ontogenesis without detracting from the pathologic features. A discussion of the pathologic features will be undertaken and an endeavor made to correlate the development of the ameloblastoma with that of the normal enamel organ.

HISTOLOGY

That the ameloblastoma is essentially an epithelial tumor with a structure resembling that of the enamel organ has been agreed by many writers since the original demonstration of the tumor by Broca [1] in 1868. Ameloblastomas are usually classed as solid or cystic. The former consist of epithelial strands in a loose connective tissue stroma. Many ameloblastomas are intermediate between these forms. Keratinization is sometimes encountered, as in sixteen of the reviewed cases, and spicules of bone may also be found occasionally. Enamel is never found in the ameloblastoma, for reasons to be pointed out later. However, two articles have reported enamel alone (Bernays,[2] Chibret [3]), one a calcified mass (Mummery and Pitts [4]), one enamel, dentin and cementum (D'Aunoy and Zoeller [5]) and one a substance suggesting the matrix of enamel (Ghosh [6]), in tumors the authors believed to be amelo-

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1. Broca, P.: Gaz. hebd. d. sc. méd. de Bordeaux **25**:70, 1868.
2. Bernays, A. C.: M. Rec. **28**:1, 1885.
3. Chibret, A.: Arch. de méd. exper. et d'anat. path. **6**:278, 1894.
4. Mummery, J. H., and Pitts, A. T.: Brit. Dent. J. **47**:121, 1926.
5. D'Aunoy, R., and Zoeller, A.: M. J. & Rec. **130**:274, 1929.
6. Ghosh, L. S.: Am. J. Path. **10**:773, 1934.

blastomas. A melanotic ameloblastoma (Mummery and Pitts) has been described. Churchill [7] in his classification recognized practically all the forms of ameloblastoma. He divided them into solid, cystic and melanotic, with or without epithelial pearl formation, and, further, recognized the malignant structure under the terms "carcinoma ameloblasticum" and "sarcoma ameloblasticum." This grouping covers the varieties fairly completely and is of some value to the clinician.

Most of the general histologic features of ameloblastomas were represented in the cases reported in this paper. Pathologic descriptions appear in subsequent sections of this paper. In case 4 there was a solid ameloblastoma in which an epithelial structure almost identical with that seen in the developing tooth was contained in an embryonic connective tissue stroma. This is evident in the photomicrographs (fig. 1 *A* and *B* and fig. 3). In case 3 was seen an ameloblastoma of the solid type, with a structure somewhat suggestive of a basal cell carcinoma (fig. 2 *A*). A transitional stage between the solid and the cystic type, with ameloblast-like cells still evident, was presented in case 2 (fig. 2 *B*). Keratinization was seen with the formation of epithelial pearls. Case 1 was an example of the cystic stage (fig. 2 *C*). Churchill has seen these sections, and considering them from the standpoint of differentiation from the dentigerous cyst he placed case 2 in the latter category. He was also somewhat doubtful as to the exact diagnosis in case 1 and suggested the possibility that the tumor was a mixed growth in an unusual situation.

Since the picture in ameloblastoma so closely resembles that of the developing enamel organ, a brief review of the embryology of the tooth will assist in pointing out these similarities. A knowledge of the history of the developing tooth is also invaluable in diagnosing an ameloblastoma and differentiating it from a dentigerous cyst and from a carcinoma. The formation of a tooth begins with a thickening of the oral epithelium at some place along the alveolar ridge. From this area of thickened epithelium the primitive dental lamina, and then the true dental lamina, is formed. In the course of development the tooth germ makes its appearance as an undifferentiated knot of epithelial cells; these cells begin to differentiate, with occurrence of a columnar type externally (fig. 1 *A1*) and subsequent evolution of the enamel organ. This bell-shaped structure (fig. 1 *A3*) consists of epithelial cells which have differentiated into four types: the external epithelial layer, the stellate reticulum, the stratum intermedium and the internal epithelial, or ameloblastic, layer (fig. 1 *A2*). As the enamel organ is formed, the cells between

7. Churchill, H. R.: Beiträge zur Klassifizierung der Tumoren Mundes und seiner Nebenorgane, Inaug. Dissert, Rostock, Carl Hinstorffs Hofbuchdruckerei, 1932.

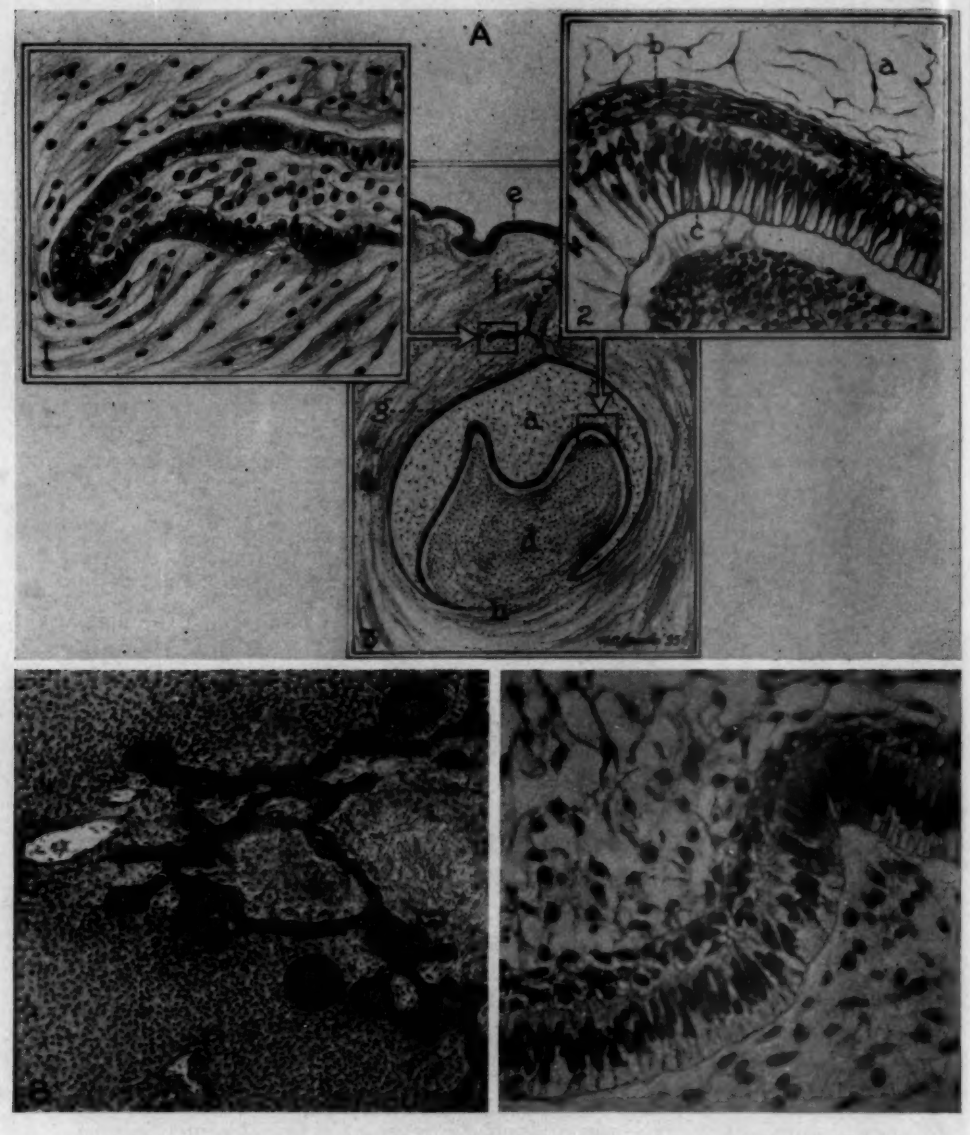

Fig. 1.—*A*, camera lucida drawings of developing tooth germs from a 3 month embryo: *1*, enamel bud of permanent tooth; *2*, enlargement at the ameloblastic layer of the deciduous tooth bud ([*a*] stellate reticulum, [*b*] stratum intermedium, [*c*] ameloblastic layer and [*d*] mesodermal tissue, the dentin papilla); *3*, enamel bud and enamel organ in situ ([*a*] stellate reticulum, [*d*] dentin papilla, [*e*] oral mucosa, [*f*] remnants of the dental lamina, [*g*] external epithelial layer of the enamel organ and [*h*] beginning of sheath of Hertwig).

B, area from a solid ameloblastoma (case 4). The similarity of the epithelial strands to the enamel bud of the normal enamel organ is notable. The epithelial tissue is in a stroma of connective tissue of the embryonic-type; × 100; hematoxylin and eosin stain.

C, area from the same ameloblastoma at a higher magnification, showing striking homology to the area of the enamel organ shown in figure 1 *A 2* (taken from section in fig. 3 *A*). The stellate reticulum-like cells, stratum intermedium-like cells and ameloblastoma-like cells are apparent. There is a noticeable scalloping of the outer edge of the ameloblast-like layer, suggesting the scalloped dento-enamel junction in the normal tooth. The nuclei of these cells are in a position similar to that of the nuclei of the normal tooth germ just before assuming function; × 430; hematoxylin and eosin stain.

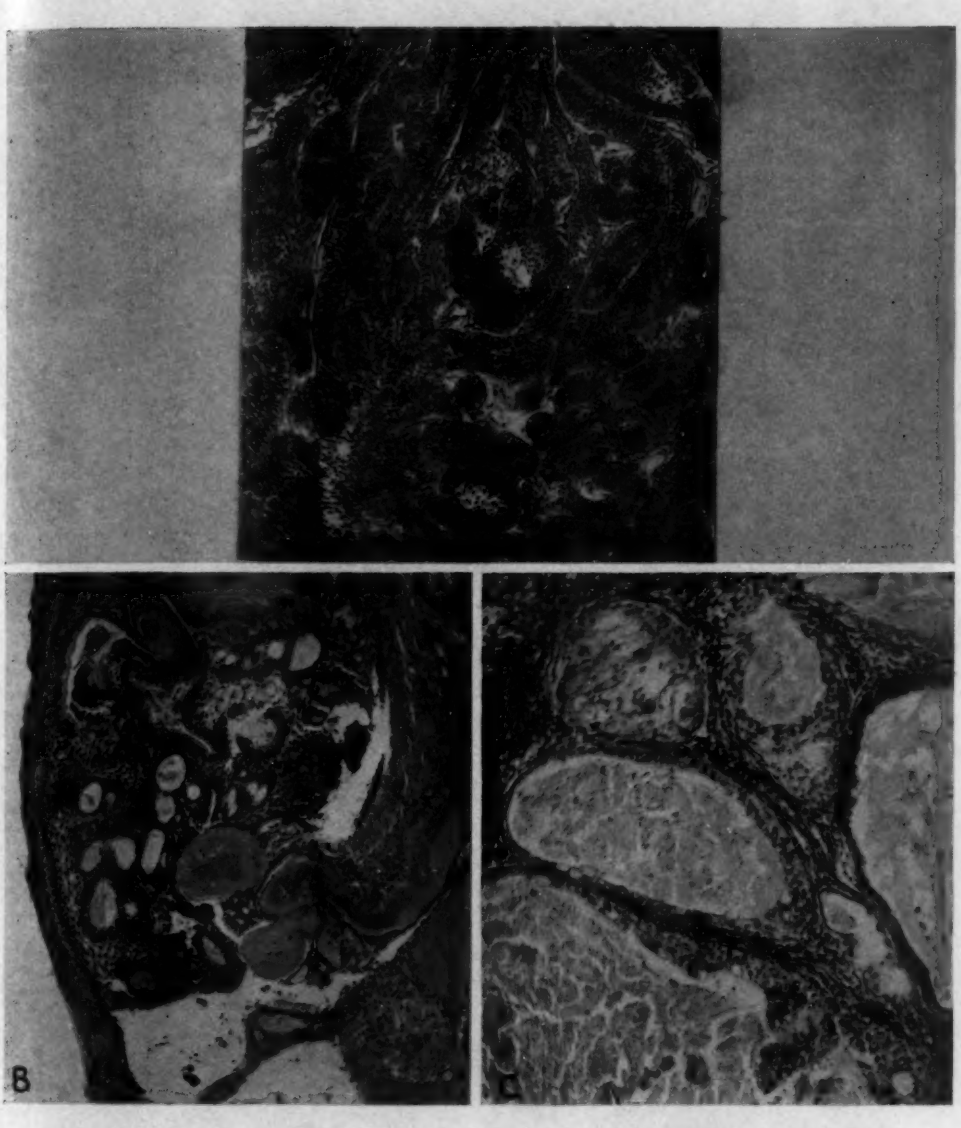

Fig. 2.—*A*, area from a solid ameloblastoma (case 3). Strands of epithelium run through a compressed stroma. In places cystic development is seen in the centers of the epithelial strands. The cells are not as definitely suggestive of those of the normal enamel bud as those in the previous illustrations, but a definite columnar form is noted externally, and the internal cells tend to separate. This is a solid ameloblastoma of the type that has been compared with the basal cell carcinoma; × 100; hematoxylin and eosin stain.

B, area from a cystic and solid ameloblastoma (case 2). The growth has passed the normal functional point and is degenerating, with formation of cysts. The ameloblast-like cells are still visible, lining the cysts. Some epithelial pearl formation is seen. This is a transitional stage; × 100; hematoxylin and eosin stain.

C, area from a cystic ameloblastoma (case 1). The ameloblast-like cells have been compressed to cuboid or even squamous form; the stellate reticulum has practically disappeared from all areas, and the centers of the cystic cavities are filled with an amorphous hyaline material with some suggestion of ghost cells. The stroma is much compressed; × 100; hematoxylin and eosin stain.

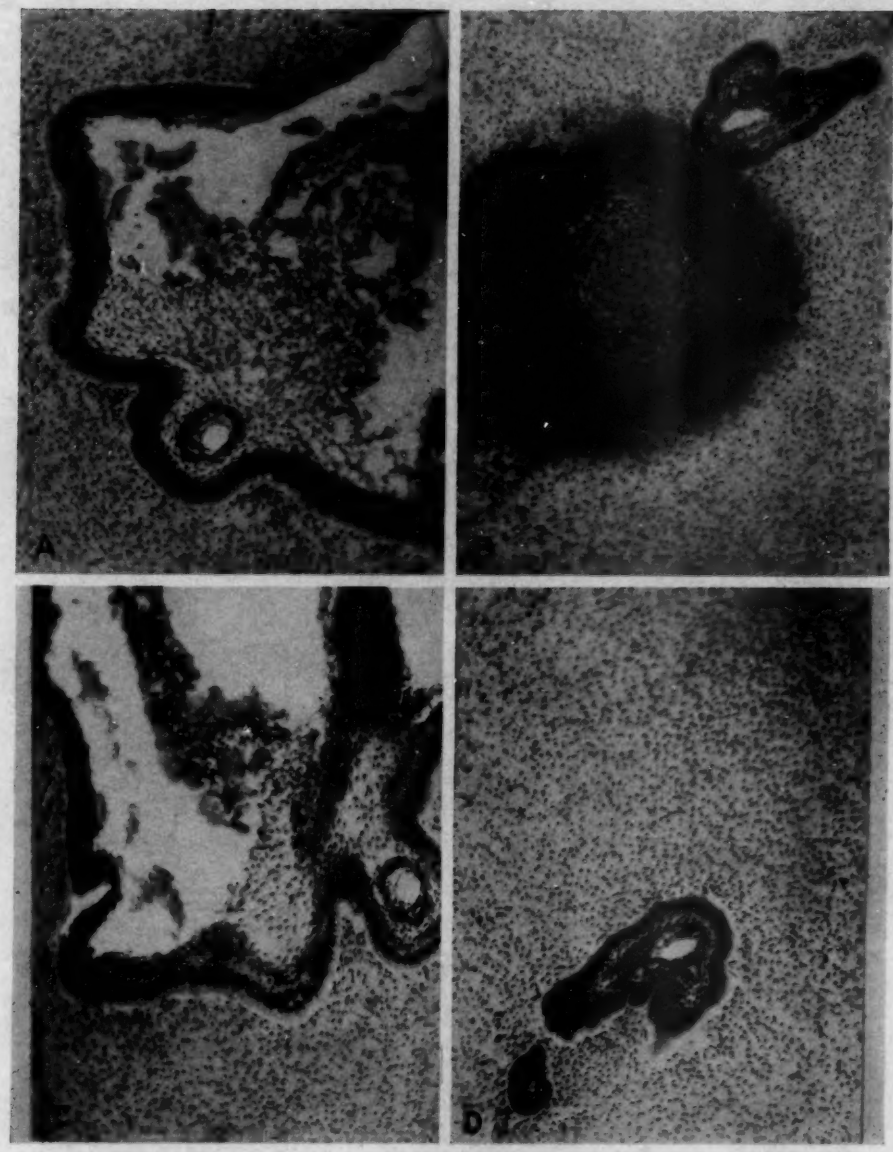

Fig. 3.—Serial sections from a solid ameloblastoma (case 4). The sections show fusion and crossing of strands. In section *B* the ameloblast-like layer is seen cut transversely.

the external and internal epithelial layers become separated, and fine protoplasmic processes extend from cell to cell, giving them a starlike appearance. The cells of the internal epithelial layer become greatly elongated, and it is these specialized cells, the ameloblasts, that function in the formation of enamel. It has been pointed out by Kronfeld [8] that ". . . for the formation of enamel, specialized mesodermal cells are necessary, for among the dental tumors we find enamel formation only among the odontoma." Apparently, before the ectodermal ameloblasts can begin forming enamel, a certain amount of dentin must be laid down by the mesodermal odontoblasts, and the ameloblast-like cells do not have the ability to activate the surrounding connective tissue to odontoblastic differentiation. It is at this point in development that the enamel organ and the enamel organ neoplasm diverge, for the enamel organ contains, between its alae, mesodermal tissue which forms dentin, but the ameloblastoma does not show development of odontoblasts, which must precede the ameloblasts in function. The similarity of structure between the enamel organ and the ameloblastoma at different early stages of their development and their divergence in structure beyond the point of the formation of enamel have been demonstrated previously (Kronfeld). However, these observations seem to be of sufficient importance and interest to warrant confirmation and expansion with a different series of ameloblastomas.

In figure 1 *B* is illustrated an ameloblastoma the structure of which corresponds to a tooth in an early stage of formation, being homologous with the dental lamina and epithelial bud (fig. 1 *A3f* and *1*). The epithelial cells of the tumor vary from oval to cuboid much as in the dental lamina and have not yet shown evidence of differentiation into the structures of the enamel organ. However, in places a stellate reticulum-like structure is seen forming between the separating external layers, much as in the normal tooth germ. Figure 1 *C* is a striking illustration of how closely the ameloblastoma may simulate the normal tooth germ. By comparing this figure with figure 1 *A2*, one may readily recognize the homologs of the ameloblastic layer, the stratum intermedium and the stellate reticulum. Figure 3 shows different areas in serial sections through this same tumor and illustrates how the follicles interlace and converge. A section from another solid ameloblastoma (fig. 2 *A*) shows that the external cells are somewhat more elongated than in figure 1 *A* but that the stellate reticulum is not as striking as in figure 1 *B*. The cells between the external epithelial layers have begun to degenerate and present ill defined, ghost cell outlines. This is the earliest sign of cyst formation, and it is apparently at

8. Kronfeld, R.: J. Am. Dent. A. **17**:681, 1930.

the expense of the stellate cells. A section from a third case (fig. 2*B*) shows a more advanced stage of cystic degeneration, with the tall ameloblast-like cells still distinct but the place occupied by stellate reticulum in the solid stage filled now with a degenerated mucoid mass. The stroma is much compressed and in places shows hyalinization and a tendency toward bone formation. Each of the follicles now represents a cyst or, in this particular case, an epithelial pearl, as some of the follicles have keratinized. In figure 2*C* a section from an advanced cystic ameloblastoma is seen. All but a few traces of stellate cells is lost in the cystic contents. The follicles have degenerated to cysts and in expanding have compressed the epithelial lining cells from their tall columnar form to a cuboid or even squamous form, although in a few places the ameloblast-like cells are still visible. The stroma is compressed and compact in contrast with the loose, embryonic-like structure of the connective tissue seen in the first section. The entire structure is far removed from the structure of the normal enamel organ, but it seems to represent a degenerated form of the growth that was seen in the chronologically young ameloblastoma.

In view of the similarity between this tumor and the enamel-forming tissues, it is suggested that a parallel development takes place up to the point at which the normal ameloblasts assume function. From this point on a divergent development can be traced, degenerate in the tumor and generative in the enamel organ, resulting in the formation of cysts, on one hand, and in the formation of enamel, on the other. While it has not been possible to follow the process through in any individual case, both Kronfeld's and these findings in a series of ameloblastomas indicate strongly that solid and cystic ameloblastomas probably represent stages of development rather than distinct types of enamel organ tumors.

REPORT OF CASES

These cases were collected from the Strong Memorial Hospital and the School of Medicine and Dentistry of the University of Rochester and occurred in patients of Dr. J. J. Morton, professor of surgery, and Dr. W. R. J. Wallace, consultant in dental surgery.

CASE 1.—*History.*—A woman 28 years of age, white, married, who had had four pregnancies and one miscarriage, stated that four months previous to admission, the last remaining lower right molar, which was in a pyorrheal area, was removed and that since that time the gum had remained sore locally. The gum had been incised several times by a local physician to relieve this soreness. In March 1930, a cystlike mass was removed and reported pathologically as "a radicular dentigerous cyst." In August of the same year, two bean-sized masses were removed and reported as "granulation tissue with tiny cysts; there is a question of tumor, possibly endothelial." In August 1932, the patient was again operated on, and the pathologic report after study of the two shot-sized

masses removed was "a radiculodental cyst of the jaw with subacute inflammation of the connective tissue." Since the pain persisted, the possibility of neuralgia was considered and, in January 1933, an injection of alcohol was given to anesthetize the third division of the trigeminal nerve. The pain returned in five weeks, and the patient was referred to the State Institute for the Study of Malignant Disease, Buffalo. After an examination in which "a very tender area in the lower jaw which appears as though it may be of adamantinomatous nature on palpation" was found, the area was curetted, but insufficient material for histologic examination was obtained. The following day the pain disappeared, and the patient was discharged without further treatment. In November 1934 she returned to the Strong Memorial Hospital with pain and swelling. The gum over the area was excised, and the cortical bone beneath, which appeared jagged and rough, was smoothed off. The patient was discharged. In January 1935 she reported no pain except on yawning.

Roentgen Observation.—At the time of the first admission lateral views showed a massive defect in the right mandible. By February 1932 the bone appeared considerably regenerated. In March 1933 the State Institute for the Study of Malignant Disease reported from roentgenograms "an appearance of osteomyelitis." Little change was noticed in plates made in November 1934.

Pathologic Observations.—The early sections showed a picture atypical of ameloblastoma. The section from the operation of March 1930 consisted of granulation tissue with cystlike cavities, lined by epithelium of an irregular type. Within the epithelial lining microscopic cysts were found. Within the cysts cholesterol clefts were numerous, and hemorrhage was found in the tissue but not in the cysts. In the section made from the tissue obtained in August 1930 a mass of granulation tissue was seen with tiny cysts of varying sizes as well as strands of epithelium. In places hyaline degeneration was apparent in the connective tissue. The section made after the operation of February 1934 showed connective tissue with scattered inflammatory cells and the cyst with irregular epithelial lining and polyps extending from the lining. A more typical picture was seen in the section obtained at operation in November 1934, namely, numerous large and small cysts lying in a dense hyalinized connective tissue stroma beneath hyperplastic oral epithelium. The cysts were lined by epithelium varying from tall columnar through cuboid to squamous. Along the margins of the cysts ghost cells suggestive of stellate reticulum could be occasionally seen. Within the cyst cavities was a pink-staining hyaline material. Sheets, strands and cords of epithelium ran through the section. Round cells and a few mitotic figures were recognized (fig. 2*C*).

NOTE.—In May 1935 the patient was admitted with slight facial swelling, tenderness and return of pain. Roentgen examination showed little change in the general appearance of the mandibular bone, but one small radiolucent area was seen in the body and another at the ramus of the bone. These two cavities were removed by chiseling and curettage. The material removed was unsuitable for histologic examination.

CASE 2.—*History.*—A woman 29 years of age, white, unmarried, stated that fourteen years prior to admission she had undergone operation for removal of a cyst in the right maxilla. One year prior to admission a small lump was noticed in this area, and a dentist was able to express pus from it. All the teeth in the region of this swelling had been removed some time before. On the buccal side of the right maxillary ridge there was a small hard tender inflamed swelling about 1.5 to 2 cm. in diameter. The patient had not been wearing her denture,

owing to painful pressure. In September 1933, a cyst was removed in toto with a curet, and the bone was loosely packed. When the patient returned a year later for excision of an unrelated tumor of the breast, no signs of recurrence were seen.

Pathologic Observations.—A section from this growth was made up of spicules of bone and cysts within a connective tissue stroma. The cysts showed various degenerated stages of ameloblastoma. In some, tall columnar cells still lined the walls, and stellate reticulum was found within. Others showed an epithelial lining the cells of which were more cuboid, and a hyaline mass within. Still others were lined with cells of the squamous type. In some areas keratinization with the formation of epithelial pearls was evidenced. The spicules of bone were found only in the connective tissue and not within the cyst walls or cavities. Hyaline degeneration and round cell infiltration were found in the stroma (fig. 2*B*).

Case 3.—*History.*—A man 49 years of age, married, said that about one year prior to admission he had noticed a hard painless nodule, on the buccal surface of the right mandibular ridge. It grew slowly until about two months before presentation, when it suddely became swollen and painful. Two days later a bloody discharge was noticed about the mandibular right second molar tooth. This disturbance was repeated in about two weeks' time. The mandibular right molar teeth were extracted. On admission the growth appeared as a nontender painless hard nodule. A biopsy was made, and the growth was reported as an ameloblastoma. At operation the cyst was worked free from the bone, care being taken to preserve the structure of the bone. The tumor mass was split and delivered in small pieces. The area was curetted and the inferior dental artery ligated. The cavity was cauterized with phenol and washed with alcohol.

Pathologic Observations.—In gross, several small pieces of tissue of irregular shape were seen, with fragments of adhering bone. The cut surface was smooth, glistening, dense and white, with several smooth red areas, which probably represented cysts. Microscopically, columns of epithelium were found in a connective tissue stroma. The epithelium was of the columnar type, with a delicate basement membrane and more irregular epithelial cells between. In places the intercolumnar cells suggested stellate reticulum, and in still other areas small cysts containing a homogeneous pink-staining material were found. While epithelial pearls were not seen, the appearance of some of the cysts suggested a tendency toward keratinization. The connective tissue showed myxomatous areas and round and plasma cells. The epithelium of the neoplasm showed connection with the oral mucous membrane (fig. 2*A*).

Case 4.—This case of an ameloblastoma in a 2½ year old girl is to be reported by Dr. J. J. Morton and Dr. W. R. J. Wallace. For the sake of clarity in consideration of the pathologic observations, this brief summary is presented. The duration was seven months. The age of the patient at the time of the discovery was 2 years. The site was the left mandible. The tumor measured 6.5 by 4 by 3 cm. In previous treatment it had been incised two or three times. The roentgen examination showed a monocystic cavity extending from the canine tooth to the ramus, with the teeth displaced. The mass was dissected out, and the deciduous canine, first molar, second molar and permanent first molar teeth were removed. For the microscopic picture see figures 1 *B* and *C* and figure 3.

COMMENT

While no single ameloblastoma has yet been traced from the solid to the cystic form through histologic studies, it seems highly possible that such a transition takes place. The solid ameloblastoma closely resembles the developing tooth up to the point at which generative processes should begin. From this point on, a graded series of degenerative changes with cyst formation in the stellate reticulum can be noted in the neoplasm. This degeneration from solid to cystic was noted by Kronfeld (1930) in a series of ameloblastomas, and his observation is confirmed by the series at hand. While this evidence is highly suggestive, it cannot be claimed that such a process is known to take place.

SUMMARY

Cystic, cystic and solid, and solid forms of ameloblastoma are encountered. Cornification, hornification or epithelial pearl formation may occasionally be found in the ameloblastoma, as in one of the cases cited and in fifteen instances collected from the literature.

The ameloblastoma morphologically resembles the normal developing enamel origin or its degenerated form. The solid, cystic and solid, and cystic forms seem to represent different stages in the degenerative transition of the tumor from solid to cystic structure.

The development of the ameloblastoma appears to parallel that of the normal enamel organ up to the point at which the latter becomes functional. From here, the enamel organ progresses to form enamel, while the enamel organ neoplasm degenerates to a nonfunctional cystic mass.

Case Reports

PRIMARY LYMPHOSARCOMA OF THE LIVER WITH METASTASES TO THE MARROW AND SECONDARY ANEMIA

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The only account of primary lymphosarcoma of the liver seems to be the report by Carl Sternberg.[1] The patient, a man aged 32 years, had indefinite pain in the upper part of the abdomen and jaundice which at first was slight but later became severe. He died after an illness of about seven weeks. The blood was not examined. The liver weighed 7,120 Gm.; the spleen, 1,120 Gm. Both contained nodules, the liver more than the spleen. Similar growths were found in both kidneys. Sternberg concluded from histologic studies that the condition was lymphosarcoma, mainly of the liver, originating on the basis of aleukemic lymphadenosis (pseudoleukemia). According to Kettler,[2] the peripheral tissues of the human liver have, independent of age, a moderate content of lymphocytes, although true lymph nodes are not usually present. This finding, of course, is entirely different from the conditions in leukemic and aleukemic lymphadenosis, in which the liver has extensive lymphocyte infiltrations. Thus, the lymphoid tissues of the liver, with or without hyperplasia, rarely become malignant.

Kundrat [3] originally stated that lymphosarcoma arises in lymphoid tissues and spreads from one group of lymph nodes to others through the lymph channels. Thaysen [4] and others, however, having observed an invasion of the intima of blood vessels in lymphosarcoma, concluded that the possibilities for hematogenous spread with these growths are as great as with other forms of sarcoma. Among 164 patients with lymphosarcoma considered in the report by Craver and Copeland,[5] 10.4 per cent had metastases in the bones. These metastases, according to the authors, arose by hematogenous spread and by direct extension from lymph nodes.

REPORT OF CASE

A white man aged 37 years entered the service of Dr. W. F. Lyon at St. Luke's Hospital, Chicago, June 1, 1936. About two years before, a progressive weakness began insidiously, and a slight jaundice appeared. After a loss of 30 pounds (13.6 Kg.) in weight during six months, he consulted a physician, and the disorder was diagnosed as pernicious anemia. The usual therapeutic treatment for this disease had no effect, and he entered the hospital for further care. His liver

From the Henry Baird Favill Laboratory of St. Luke's Hospital.
The investigation was aided by the Winfield Peck Memorial Fund.

1. Sternberg, Carl: Wien. med. Wchnschr. **84**:417, 1934.
2. Kettler, L.: Virchows Arch. f. path. Anat. **291**:706, 1934.
3. Kundrat, A. E.: Wien. klin. Wchnschr. **6**:211, 1893.
4. Thaysen, T. E. H.: Beitr. z. path. Anat. u. z. allg. Path. **50**:520, 1911.
5. Craver, L. F., and Copeland, M. M.: Arch. Surg. **28**:809, 1934.

and spleen were enlarged; the erythrocytes of the blood varied between 1,400,000 and 1,250,000, and the leukocytes between 9,850 and 6,900 per cubic millimeter; the hemoglobin ranged from 4.5 to 5.1 Gm. per hundred cubic centimeters. The red blood cells were irregular in size, shape and staining qualities. Of the leukocytes, from 51 to 61 per cent were lymphocytes, from 3 to 4 per cent monocytes, from 33 to 41 per cent morphonuclear leukocytes, and from 2 to 5 per cent band cells. The percentage of the reticulocytes varied between 0.4 and 1.7. Intensive liver therapy, advised by Dr. Joseph L. Miller, had no effect on the anemia. On June 15 death occurred suddenly from air embolism during a direct transfusion of blood (pump).

The essentials of the postmortem examination (head, neck and trunk), made immediately after death, are contained in the following paragraphs. Frothy blood was demonstrated in large quantities in the chambers of the heart and in the systemic blood vessels, even the veins. The liver weighed 2,200 Gm. The capsule was smooth, and the parenchyma beneath was mottled slightly in shades of brown.

Fig. 1.—Photograph of a surface of the right lobe of the liver, revealing periportal lymphosarcoma. No appreciable amount of the tumor tissue extended to the surface of the liver except at the hilus.

The portal vein was surrounded at the hilus by firm gray-white tissues, which extended along its divisions into the right and left lobes, more extensively into the right than into the left. These collars of gray-white tissue around the portal vein and its branches (fig. 1) were as wide as 1.3 cm. They encased the structures of the portal canals, had replaced the adjacent liver tissues, but decreased toward the periphery of the liver and did not reach the capsule. A subcapsular nodule lay in the inferior surface of the right lobe of the liver, medial to the gallbladder. The linings of the large branches of the portal vein seemed smooth, although subsequent histologic examination disclosed tumor invasion of the intima and even mural thrombi. The lining of the right and left hepatic ducts was unchanged. The biliary lymph nodes appeared as flat, gray tissues, the largest measuring 3 by 1.5 by 0.8 cm. Some of the perigastric lymph nodes were slightly enlarged. Several subpleural rounded nodules of gray-white tissue, from 1 to 1.5 cm. in diameter, were distributed near the spine, on both sides, in the upper part of the thorax. They were closely adherent to and continuous with the ribs;

the cortex of the ribs was rough. The spleen weighed 660 Gm. The pulp was dark red and had no obvious tumor tissues. Other viscera and lymph nodes had no definite metastases of the tumor, although later some small growths were demonstrated by histologic examinations.

The histologic structure of the gray tissues about the portal veins was studied in sections stained with hemtoxylin and eosin and with phosphotungstic acid-hematoxylin and by the Laidlaw method for reticulum fibers. The periportal tissues consisted mainly of small round or polygonally compressed cells with round vesicular nuclei containing fine and coarse chromatin granules. They had a little granular cytoplasm. The cells corresponded in size and appearance to small lymphocytes. A few cells were twice or thrice as large and had 2 or more nuclei.

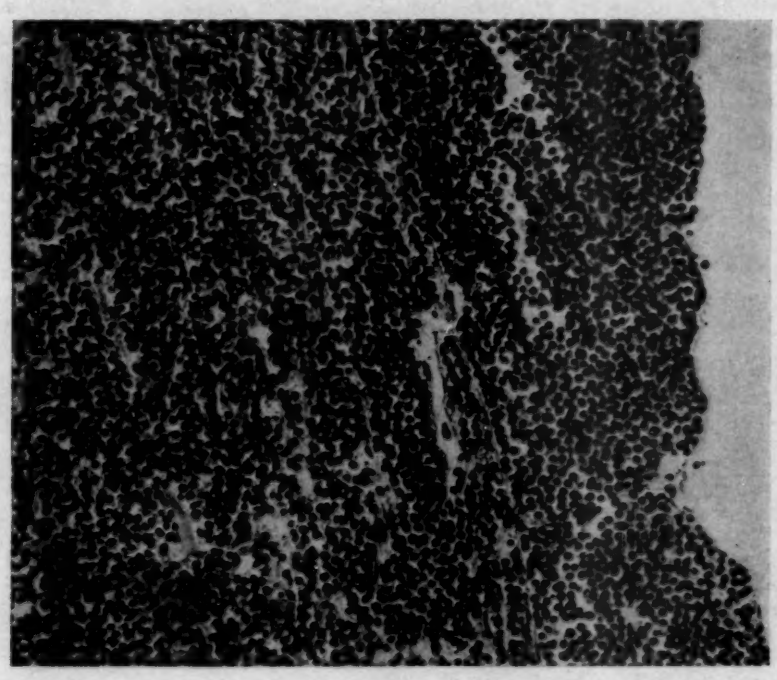

Fig. 2.—Photomicrograph of a section through the wall of a large branch of the portal vein; reduced from × 270. Note the marked invasion of the vein by lymphocytes and the mural tumor cell thrombus (at the right edge). The stroma of the new growth is scanty. Masses or single cells swept into the circulation from these thrombi provided abundantly for the metastatic spread of tumor tissues.

The collagenous stroma fibers formed small compartments containing aggregates of these cells. A few fine fibrils were between the cells. The stroma was more abundant in some portions than in others and was in close contact with the small round cells. The reticulum fibers in the Laidlaw preparations were distributed the same as the stroma in the phosphotungstic acid-hematoxylin preparations. The cells of the new growth had penetrated the walls of large and small divisions of the portal vein. The lumens of some of these vessels contained thrombi of tumor tissue. Along the intimal edge of large branches of the portal vein (fig. 2) were aggregates of tumor cells with little stroma, which could be

dislodged easily into the lumens to be swept away singly or in small groups by the blood stream. The walls of the bile ducts also were invaded by tumor cells. The tumor tissues along the periphery penetrated the sinusoids of the hepatic parenchyma, replacing them. These invasive tissues did not have the structure of granulation tissues, nor did they resemble the exudates of chronic inflammation. The marrow of the ribs examined was entirely replaced by cellular tissues with the structure of the new growth in the liver (fig. 3). These tissues filled the large and small marrow spaces and extended through the latter into the periosteum and adjacent soft tissues. There was no growth of new bone; many of the bone trabeculae were narrow as with atrophy. The conditions of the marrow in the lumbar vertebrae and in the sternum were similar. Only a few remnants

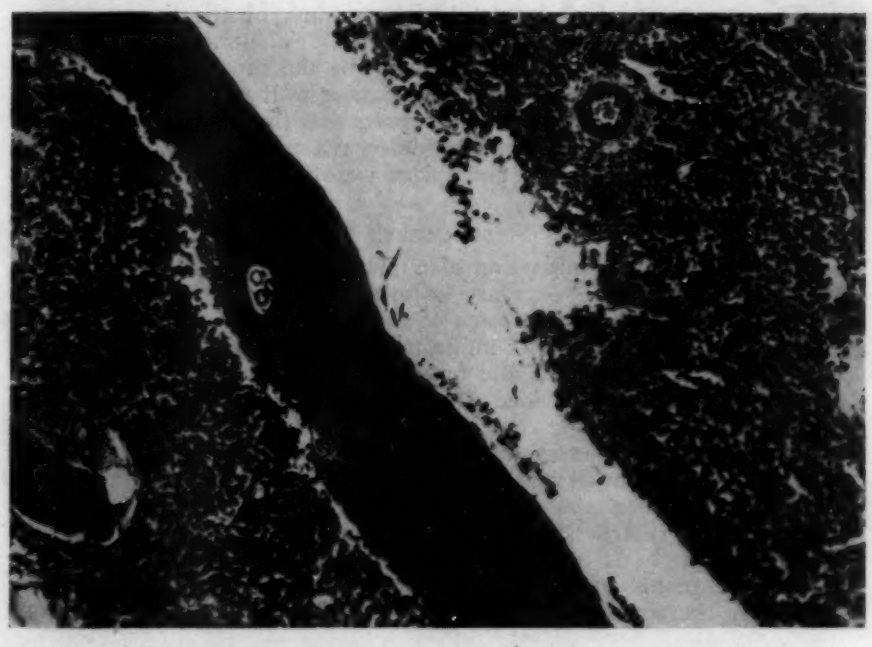

Fig. 3.—Photomicrograph demonstrating metastatic lymphosarcoma in the marrow of a rib; reduced from × 270. The portions to the right of the bone are displaced marrow tissues; those to the left are periosteal soft tissues invaded by sarcoma which had penetrated the cortex.

of the original cells of the marrow remained. The subpleural nodules along the thoracic portion of the spine were extensions of growth from the marrow of the ribs. The routine histologic examinations of the viscera demonstrated small tumor tissue infiltrations along the septums of the pancreas, into the periadrenal fat and into a perigastric lymph node, and in the spleen were some cell aggregates certainly like tumor tissues, although not forming distinct nodules.

COMMENT

The tumor tissues in the liver doubtless were the primary focus from which metastases were spread through the blood after the walls

of the portal tributaries had been invaded. The thrombi of tumor cells with scanty stroma provided abundantly for a spread of cell masses or even single cells into remote tissues by embolism. The marrow was the tissue most favorable to secondary implantation. This extensive metastatic spread of the tumor into the marrow and the consequent replacement of the hematopoietic elements by its growth probably explain the severe anemia noted clinically. Perhaps some of the cells considered lymphocytes in the differential examinations of the leukocytes were tumor cells circulating in the blood. The secondary deposits in the bones did not stimulate a growth of bone but, rather, caused slowly progressive erosion, especially in the ribs—changes in agreement with those observed by Craver and Copeland in osseous metastases of lymphosarcoma.

The symptoms of periportal lymphosarcoma of the liver are not sufficiently characteristic for clinical diagnosis. The only other account of lymphosarcoma of the liver seems to be the report by Sternberg. The advancement of the disease in his patient at the time of death was greater than in the patient of my report, and the distribution of the tumor tissues in the liver was in nodules, not periportal. Mild icterus and enlargement of the liver and spleen were present in both.

SUMMARY

The postmortem examination of a man aged 32 years whose clinical symptoms were severe secondary anemia and slight jaundice disclosed primary periportal lymphosarcoma of the liver.

The lymphosarcoma cells infiltrated the walls of large and small branches of the portal vein and produced mural and obturating tumor tissue thrombi, which provided abundantly for hematogenous spread of cell masses or even single cells into remote tissues by embolism.

Extensive metastases in the bones replaced the hematopoietic tissues. The growth of tumor tissues in the ribs penetrated the haversian canals, destroying the bone and extended into the surrounding soft tissues. The extensive destruction of the hematopoietic tissues in the bones seems to be the basis for severe anemia.

The symptoms of primary lymphosarcoma of the liver are not sufficiently characteristic to establish a clinical diagnosis of the disease.

ANEURYSM OF A SINUS OF VALSALVA WITH RUPTURE INTO THE RIGHT AURICLE

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Not infrequently an aneurysm of a sinus of Valsalva ruptures into the pericardium, mediastinum, left auricle, pulmonary artery or superior vena cava, but only in three cases has rupture into the right auricle been found (Dahlen,[1] Charcot,[2] Higgins[3]).

REPORT OF A CASE

A colored chauffeur, 27 years old, was admitted to the service of Dr. Harry M. Stein, Jan. 27, 1936. His complaints were shortness of breath, pain in the lower part of the stomach and pain under the ribs.

Eight days before admission, while driving a car, he experienced a sudden onset of dyspnea with constant dull nonradiating pain under the sternum, a sense of swelling of the abdomen, a constant dull, sometimes sharp, pain in both lower quadrants of the abdomen, and a pain below the right costal margin on slight pressure. These symptoms increased in severity until admission.

The patient was well developed, not jaundiced or cyanotic, but orthopneic, and he coughed small clots of blood. The veins of the neck were prominent. Fine crackling râles were present at both bases of the lungs posteriorly. The apex of the heart was 13 cm. to the left, in the sixth interspace. There was a definite thrill in the pulmonary region. At the mitral area there was a systolic and diastolic murmur that obscured the sounds. The second pulmonic sound was accentuated. The blood pressure was 140 systolic and from 20 to 0 diastolic. The pulse was of the water-hammer (Corrigan's) type, regular in force and rhythm, and the rate, 120 per minute.

A typical pistol shot sound was heard over the femoral artery. The abdomen was rounded and soft. The liver was 3 fingerbreadths below the costal margin and tender. The urine showed a trace of sugar and of albumin, and the Wassermann reaction of the blood was four plus.

The diagnosis was syphilis of the aorta with aortic insufficiency, myocardial failure and chronic passive congestion.

The patient died March 5.

Autopsy.—The body was that of a well developed and well nourished colored man. External examination showed nothing of importance.

The left pleural sac contained about 200 cc. of a clear serous fluid, while the right was empty. There were many coarse adhesions over the posterior portions of both lungs. The lungs contained air throughout. The cut surface showed numerous small dark red hemorrhages.

The pericardial sac contained about 60 cc. of clear serous fluid. There were no adhesions.

From St. Joseph's Hospital and the Department of Pathology of the University of Maryland.

1. Dahlen, B.: Ztschr. f. klin. Med. **63**:163, 1907.

2. Charcot, quoted by Dahlen.[1]

3. Higgins, A. R.: U. S. Nav. M. Bull. **32**:47, 1934.

The heart weighed 620 Gm. The epicardium was smooth. The right and left ventricular walls were greatly thickened, and their architecture was coarse. There was a thrombus in the left auricular appendage. The tricuspid, pulmonic and mitral valves appeared normal. Two of the aortic cusps showed slight thickening, while the right cusp was extremely thick and contracted. The endocardium below this cusp was white and thickened. This scarring extended so as to involve slightly the left cusp. Behind the thick, contracted cusp there was a deep pocket, which led downward and opened into the right auricle a short distance above the tricuspid valve. The aortic end of this somewhat irregular but roughly conical sac was about 1 cm. in diameter, while the auricular opening was 4 mm. in diameter. The auricular opening was at the top of a rounded elevation which projected into the right auricle. The edge of the opening was round and smooth. Both coronary arteries had normal-appearing ostia. The aorta showed no other gross evidence of syphilis.

The liver was greatly enlarged and smooth. There was marked congestion with typical nutmeg mottling.

The spleen, pancreas, gastro-intestinal tract, adrenal glands, kidneys, bladder and prostate gland appeared normal.

The brain was not examined.

Microscopic Notes.—The myocardial cells of the left ventricle had large deep-staining nuclei and granular cytoplasm. The left auricle showed considerable congestion and foci of small round cells, which were closely associated with a thrombus that was undergoing organization. There were a few small foci of round cells in the epicardium over the auricle.

The aorta disclosed marked perivascular infiltration of the adventitia by small round cells. This followed the vessels into the media, where it was not so pronounced. The intima showed no definite abnormality.

There was marked active congestion of the lungs, with a small amount of blood in the alveoli. There was no evidence of pneumonia or of definite chronic passive congestion.

The sinuses of the spleen were large and filled with blood. There were no necroses.

There were marked central congestion and necrosis in the lobules of the liver. About half of each lobule was destroyed. The portal areas appeared normal. There were no necroses, hemorrhages or infiltrations in the kidneys. Bowman's capsule and some of the tubules contained small quantities of granular albuminous material. The epithelium of the convoluted tubules was swollen and granular. There was moderate congestion.

Anatomic Diagnosis.—Syphilis of the aorta and aortic valve; cardiac hypertrophy and dilatation; aneurysm of a sinus of Valsalva with rupture into the right auricle; active pulmonary congestion; chronic passive congestion of the liver; hydrothorax on the left; hydropericardium; pleural adhesions.

SUMMARY

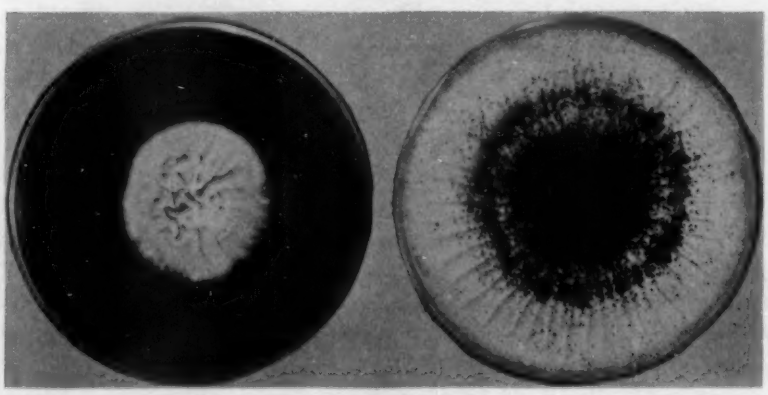
This is a report of a case of aneurysm of a sinus of Valsalva with rupture into the right auricle forty-six days before death from congestive heart failure. The absence of cyanosis, the presence of active pulmonary congestion, the absence of chronic passive congestion of the lungs and the presence of chronic passive congestion of the liver are of special interest and easily understood from the type of lesion found.

Laboratory Methods and Technical Notes

PREPARATION OF PERMANENT GROSS COLONIES OF FUNGI FOR TEACHING PURPOSES

ANDREW WALLHAUSER, M.D., PITTSBURGH

The use of permanent specimens of giant colonies of the various fungi has been found valuable in the teaching of mycology. Usually it is necessary to prepare the living specimens several weeks ahead of the time for exhibition. This necessitates a planting schedule based on a full knowledge of each organism's rate of growth, special requirements and eccentricities. To obviate this somewhat complicated procedure,



At the left, cerebriform colony of Epidermophyton cruris after one month's growth. At the right, colony of Aspergillus niger after twelve days' growth, showing individual heads and rings.

a technic for the preparation of permanent museum specimens has been developed with quite successful results as to the preservation of both color and form. The advantages of a collection of such permanent specimens lie not only in its value in the classroom but in its offering a ready means for the preliminary identification of many fungi.

The fungus is inoculated carefully in the center of a Petri dish containing the chosen solid medium. The amount of medium used should be minimal to facilitate the final drying process, a depth of ⅛ inch (0.32 cm.) being usually sufficient. The plates are inverted as usual and incubated under optimal conditions for the specified organism.

After a suitable growth has been obtained, the Petri dish is opened, and 5 cc. of solution of formaldehyde U. S. P., full strength, is placed in the cover.

From the Department of Bacteriology and Pathology, University of Pittsburgh.

The dish is again closed in the inverted position, the cover being below. The formaldehyde liberated by the solution is allowed to act for a period of from four to seven days. The solution is then removed, the cover dried and replaced. Slow drying at room temperature is then carried out for about three weeks. The cover is then cleaned, and the Petri dish is sealed by ringing the edge of the lower part with a petrolatum-paraffin mixture. The preparation being completed, it is labeled and stored in a darkened closet until required.

Specimens prepared by this method have remained well preserved for over seven years and have been found to be of practical value.

General Review

RÔLE OF VITAMIN C IN RESISTANCE

DAVID PERLA, M.D.
AND
JESSIE MARMORSTON, M.D.
NEW YORK

(Concluded from page 575)

VIII. EFFECT OF INSUFFICIENCY OF VITAMIN C ON ACQUIRED RESISTANCE TO EXPERIMENTALLY INDUCED INFECTION

Mycobacterium Tuberculosis.—The effect of chronic latent scurvy on the course of an existing infection with Myco. tuberculosis has been investigated.

The acquired resistance of tuberculous guinea-pigs that were well fed except in regard to vitamin C was in general considerably less than that of normally fed infected animals of almost any age, according to Bieling.[77] It was not the undernutrition itself, he maintained, which was the factor in early death but the absence of vitamin C in the diet, for the depression in resistance was observed even if the guinea-pig remained constant in weight. In guinea-pigs with chronic tuberculosis the effects of withdrawal of vitamin C were more pronounced than in the uninfected. He observed no disturbance in the consumption of oxygen in scorbutic animals as determined by the Benedict apparatus.

Many of his guinea-pigs had chronic tuberculosis of two years' duration. Within from five to nine days after such animals were placed on a scorbutic diet, they died. It seemed unlikely that death occurring in so short a period was the result of a break in the mechanism of resistance with reactivation of the tuberculous process. At autopsy no evidence of fresh miliary dissemination of tubercles was noted, so that the tuberculous infection did not apparently flare up under the influence of the deficient diet. There was no sudden increase in the virulence of the tubercle bacilli. The possibility of the effect of toxemia either from the tuberculous process or from the undernutrition was excluded, as no symptoms of this were present. Chronically tuberculous guinea-pigs died before any symptoms of scurvy developed when such animals were placed on a diet deficient in vitamin C. These observations may explain in part the increased mortality

77. Bieling, R.: (*a*) Ztschr. f. Hyg. u. Infektionskr. **101**:442, 1923; (*b*) **102**:568, 1924.

from tuberculous infection in human beings subjected to conditions of undernutrition.

It is regrettable that the data on which these deductions are based are so limited and so inadequately presented. They are concerned chiefly with the effect of a scorbutic diet on the course of a tuberculous infection induced with small quantities of virulent tubercle bacilli some time prior to the change in diet. The infection existed for periods of from one to two years prior to the alteration in diet. Apparently, an animal suffering with chronic tuberculous infection is more susceptible to effects of deprivation of vitamin C in the diet than a noninfected animal similarly treated.

In other experiments, Bieling [77b] injected tubercle bacilli into guinea-pigs and three weeks later altered the diet of the animals from a normal one of hay, turnips, fresh grass and oats to one of oats and autoclaved milk. Another group were put on the scurvy-producing diet after six weeks of tuberculous infection. Animals for which the diet was changed had a shorter duration of life than those for which it was not altered.

The increased sensitivity to avitaminosis was more apparent after six than after three weeks. None of the animals, when they died, showed widespread tuberculosis. The experiments were restricted to small numbers of guinea-pigs, and the diets were deficient in other vitamins as well as in vitamin C. Bieling [77b] suggested that the occurrence of scurvy even in mild degree may constitute a menace to the health of the chronically tuberculous. This conclusion seems justified.

Heymann [78] studied the effect of a diet deficient in vitamin C on the course of an induced infection with tubercle bacilli in guinea-pigs. The infection was initiated before the diet was altered. The infecting dose in all instances was 0.00001 mg. of a human strain of tubercle bacilli. The diet consisted of oats, hay and water. A small but inadequate quantity of lemon juice was added to the diet. In guinea-pigs infected with tubercle bacilli from three to four weeks prior to the change in diet no change in the course of the tuberculosis occurred, nor did evidence of scurvy develop. When in a second experiment the quantity of lemon juice was half the amount and other conditions as in the first experiment, the animals soon lost weight, showed signs of scurvy and died within an average period of approximately two months after the beginning of the experiment as compared with four and a half months in the control group. No influence on the character of the tuberculous process was noted, but there was an influence on the tempo of the infection. No tuberculosis of bones or joints was observed.

In these experiments of Heymann,[78] though the diet appears adequate in respects other than that of vitamin C, the numbers of animals

78. Heymann, B.: Klin. Wchnschr. **5**:59, 1926.

used in the various groups are not mentioned and it is difficult, therefore, to evaluate his data. Apparently, however, deficiency of vitamin C in the diet of the tuberculous animal hastens the fatal issue.

McConkey and Smith [79] studied the effect of adequate amounts of vitamin C and D in the diet on the course of intestinal tuberculosis in guinea-pigs. They fed tuberculous sputum to 72 guinea-pigs daily for periods ranging from six weeks to four months. Of 37 that were maintained on a diet partially deficient in vitamin C, 26 acquired intestinal tuberculosis. Of the other 35 animals, on a diet that was supplemented by an adequate amount of vitamin C, only 2 showed tuberculous ulcers in the intestines. From these data the conclusion seems justified that an adequate supply of vitamin C protects the tuberculous guinea-pig against ulcerative intestinal tuberculosis and that mere ingestion of tubercle bacilli by the guinea-pig is not the sole factor in the production of intestinal tuberculosis. The course of the general disease is not modified.

Analogous experiences in human beings have not been collected. Apparently, deficiency in vitamin C leads to alterations in the permeability of the intestinal mucosa, as was indicated from the earlier observations of McCarrison.[33] The high incidence of tuberculous enteritis, particularly in women, at institutions such as Montefiore Hospital, where diets adequate in vitamin C are given, suggests that perhaps in man another mechanism is operative. This problem is now being studied in the tuberculosis division. Many of the patients enter the country sanatorium early in their pulmonary disease, and intestinal tuberculosis develops while they are under observation. It may, however, be that functional digestive disturbances interfere with adequate absorption of vitamin C.

Summary.—If guinea-pigs are fed a diet containing insufficient quantities of vitamin C but not completely depleted of this factor, a chronic infection of relatively low virulence may run a more rapid and severe course in them than in normally fed animals. Guinea-pigs that have suffered with tuberculous infection of a low grade for long periods of time may when placed on a diet deficient in vitamin C die rapidly, before manifestations of scurvy appear. Their death may be due either to sudden progression of the tuberculosis (Heymann [78]) or to toxemia induced by the avitaminosis. Undernutrition itself is not responsible for this drop in resistance.

An insufficient quantity of vitamin C in the diet increases the incidence of intestinal tuberculosis in guinea-pigs previously inoculated with virulent tubercle bacilli and subsequently fed tuberculous material.

79. McConkey, M., and Smith, D. T.: J. Exper. Med. **58**:503, 1933.

These experiments are concerned more with disturbances in *acquired* resistance to tuberculous infection.

Apparently, the acquired resistance of the guinea-pig established by an induced infection with Myco. tuberculosis of low virulence is lowered if the diet is subsequently depleted partially or completely of vitamin C. The tempo of the disease increases, or the animals die of acute toxemia.

IX. EFFECT OF EXCESS OF VITAMIN C ON NATURAL RESISTANCE TO TOXINS, SPONTANEOUS INFECTIONS AND INDUCED INFECTIONS

The growing interest in the importance of vitamins in the diet in the bodily well-being, and the accumulation of evidence of the intimate dependence of natural resistance to infection on dietary factors, have led investigators to test the value of an excess of such factors on resistance. Much of this work has been disappointing. It may be that deficiency of a factor essential to normal cellular metabolism results in depression of the organism to abnormal stimuli, as infection, toxemia and the like. It does not necessarily follow that an excess above the bodily requirements increases the natural resistance of the host. That is, there is probably an optimal effect of any nutritional factor, and an excessive intake may not result in raising the optimal effect. However, in the case of vitamin C, sufficient evidence has accumulated to suggest the importance of cevitamic acid in oxidation-reduction processes in cellular metabolism, and it is conceivable that increase in its availability raises the capacity of the tissues to destroy toxic substances introduced into the body. On the other hand, certain dangers in experiments of this type should be emphasized. The animals used should be of the same stock, the history of which should be known. The dietary regimen of the mother as well as of the experimental animal since birth should be under control and adequate in vitamin content. Relative deficiency during the early months of life may cause permanent impairment in resistance regardless of a subsequent normal diet. A rise in the natural resistance of a group of animals given a vitamin in excess, above that of a control group, may not indicate an increase above the normal optimum but may suggest that a relative deficiency of the vitamin had previously existed in both groups. With these points in mind, it is difficult to evaluate the data in this field. Is one certain of the optimal vitamin requirements? Can the criteria of growth, progressive increase in weight and absence of clinical evidences of deficiency be accepted as adequate? Is it not possible that all these may be present and still, under a given stress, such as invasion with micro-organisms or injections of poisons, the apparent optimal amounts do not prove to be so?

For these reasons it is difficult to apply the implications of experimental evidence of effects of a temporary excess of a vitamin on resistance in animals of unknown source directly to human infection. In view of these remarks, the least one may demand of experiments of this type is, first, rigid control of the stock from which the experimental animals are drawn; second, the use of a strain of animals raised under ideal laboratory conditions, with a constant complete dietary for more than one generation; third, adequate controls in sufficient numbers to eliminate chance variations; fourth, statistically significant differences between the results in the group given the excess vitamin and the controls.

Infections: Tuberculosis.—Heise and Martin [80] showed experimentally in guinea-pigs that the injection of a great excess of crystalline vitamin C for a period of five months did not alter the course of induced tuberculosis.

They gave each of 10 guinea-pigs a daily intraperitoneal injection of 20 mg. of cevitamic acid during a period of one week prior to and five months subsequent to a subcutaneous injection of 300,000 virulent human tubercle bacilli (H_{37}). Five controls were given the tuberculous infection at the same time. The total amount of cevitamic acid given to each animal was 30 Gm. No detrimental effects of the daily injections were observed. When one of the controls died, all the remaining animals were killed and autopsies made. No significant differences in the dissemination and severity of the disease in the two groups were noted.

Leichtentritt [81] attempted to increase the resistance of guinea-pigs to tuberculosis by giving them an excess of vitamin C.

The normal dietary of the guinea-pigs consisted of oats, dried milk and lemon juice (0.5 cc.) daily. In two preliminary experiments in which the animals were infected by means of sputum from a tuberculous patient, she observed a slight beneficial effect from an excess of lemon juice in the diet on the longevity of the infected animals.

In another experiment, 11 guinea-pigs were infected with the tubercle bacilli contained in sputum. Five of these each received 20 cc. of lemon juice daily in addition to the diet, for seventy-three days. Four received 20 cc. of hydrous wool fat as a control. The differences in longevity in the two groups were slightly in favor of those given additions of lemon juice. Using cerebrospinal fluid obtained from a patient with tuberculous meningitis as a source of the infecting organisms, she made a similar experiment in which 2 guinea-pigs received supplements of lemon juice and 5 received lemon juice heated to 40 C. in vacuo. Those receiving fresh lemon juice lived twice as long as the controls.

These experiments yielded data which, while suggestive, are of little value. The source of infection could not be standardized, and the

80. Heise, F. H., and Martin, G. J.: Proc. Soc. Exper. Biol. & Med. **35**:337, 1936.

81. Leichtentritt, B.: Ztschr. f. Hyg. u. Infektionskr. **102**:388, 1924.

animals were too few for one to consider that the error of chance variation had been eliminated.

In an additional experiment, 8 guinea-pigs were each infected with 0.0025 mg. of tubercle bacilli (human) subcutaneously. Of these, 6 were given daily dietary supplements of 15 cc. of lemon juice and 2 were fed an adequate diet. The two controls died at a little over two months after infection. Of the guinea-pigs fed excessive supplements of lemon juice, 2 died at four and a half and six and a half months, respectively, and the remaining 4 were killed after a period of about seven months. The latter had gained in weight and showed little tuberculosis. (The strain of tubercle bacilli used was not very virulent.)

Apparently, an excess of vitamin C may increase the resistance of guinea-pigs to tuberculous infection.

The use of high vitamin supplements to the diet has been recommended in the treatment of intestinal tuberculosis. McConkey [82] treated 128 patients with intestinal tuberculosis complicating pulmonary disease. Of these, 50 were given ultraviolet radiation; 50 were given cod liver oil and tomato juice in liberal quantities, and 28 were treated palliatively. Of those given heliotherapy, 60 per cent improved, while 68 per cent of those receiving cod liver oil and tomato juice improved. Of the control group, only 3.5 per cent improved. When, after an unstated period, the patients were traced, 71 per cent of the untreated group, 24 per cent of those given heliotherapy and only 10 per cent of those treated with vitamins were dead.

These encouraging results are difficult to evaluate. The diagnosis of intestinal tuberculosis is not easily made on the basis of symptoms. Even with the aid of roentgenograms, the diagnosis may be deceptive (Schwatt and Steinbach [83]).

Frimodt-Moller,[84] in India, gave 25 persons suffering from intestinal tuberculosis diets high in vitamins, with beneficial effects. Brown and Sampson stated that cod liver oil and tomato juice in abundance had cured many patients of intestinal tuberculosis.[84a]

Steinbach and Rosenblatt [85] fed diets high in vitamins (tomato juice and cod liver oil) to patients with advanced pulmonary disease during a period of two years without obtaining any evidence of healing of intestinal complications. Such therapeutic tests are hardly a fair indication of the usefulness of a high vitamin C content in the diet.

The evidence is only suggestive that an excess of vitamin C may increase the natural resistance of the host to tuberculosis. Neither

82. McConkey, M.: Am. Rev. Tuberc. **21**:627, 1930.

83. Schwatt, H., and Steinbach, M. M.: Am. Rev. Tuberc. **8**:9, 1923.

84. Frimodt-Moller, C.: Indian M. Gaz. **65**:692, 1930.

84a. Brown, L., and Sampson, H.: Intestinal Tuberculosis: Its Importance, Diagnosis and Treatment, Philadelphia, Lea & Febiger, 1930.

85. Steinbach, M. M., and Rosenblatt, M. B.: Am. Rev. Tuberc. **31**:35, 1935.

the experimental nor the clinical observations are unequivocal. It is possible that an excess may decrease the incidence of intestinal tuberculosis in tuberculous guinea-pigs.

Diphtheria Toxin.—Independently, Jungeblut and Zwemer [86] and Greenwald and Harde [87] studied the effects of an excess of vitamin C as cevitamic acid on diphtheria toxin in vivo and in vitro. They had the opportunity of utilizing crystalline vitamin C.

Jungeblut and Swemer [86] added to 2 M. L. D. of diphtheria toxin varying amounts of vitamin C from 0.05 to 100 mg. at a p_H of from 6.6 to 6.8. After allowing the mixtures to stand half an hour at room temperature, they injected them subcutaneously into 37 guinea-pigs. They found that from 0.5 to 5 mg. of vitamin C detoxified the toxin. Greater or lesser amounts did not. Of 22 controls given the same amounts, all died within from twenty-four to seventy-two hours showing symptoms of diphtheritic intoxication. In a second experiment, the effects of vitamin C in vivo were tried. They gave each of 14 guinea-pigs 2 M. L. D. of diphtheria toxin subcutaneously and immediately afterward injected vitamin C in acid solution in amounts varying from 1 to 200 mg. Half the guinea-pigs died, and half survived 2 M. L. D. Amounts of from 5 to 25 mg. of vitamin C were protective. Excessive amounts were toxic. In a third experiment, 13 guinea-pigs were given daily subcutaneous injections of vitamin C in varying amounts. Each was then given 4 injections of diphtheria toxin in amounts of 1/50, 1/100, 1/200, 1/500 M. L. D. intracutaneously on two sides of the abdomen. Readings of the degrees of local inflammation were made every day for five days. The extent and the degree of redness as well as the intensity of necrosis were recorded. The stored vitamin C inhibited or suppressed the local reaction to diphtheria toxin.

Jungeblut and Zwemer concluded that vitamin C inactivates diphtheria toxin and helps to protect guinea-pigs against the fatal outcome of diphtheritic intoxications. Guinea-pigs treated with suitable amounts of this substance are rendered temporarily negative or definitely less sensitive to small doses of the toxin, as determined in intracutaneous tests. They believed that the results suggest that vitamin C plays an important part in the mechanism of natural resistance to diphtheria toxin.

Similar findings were reported at an earlier date by Harde [88] and by Harde and Philippe.[89] Appearing at the same time was the report of Greenwald and Harde [87] on the effect of feeding an excess of vitamin C on the resistance to diphtheria toxin.

They fed 4 guinea-pigs an excess of spinach for ten days, and then injected 1 M. L. D. of toxin into each, and all died. Each of 5 guinea-pigs was given

86. Jungeblut, C. W., and Zwemer, R. L.: Proc. Soc. Exper. Biol. & Med. **32**:1229, 1935.

87. Greenwald, C. K., and Harde, E.: Proc. Soc. Exper. Biol. & Med. **32**: 1157, 1935.

88. Harde, E.: Compt. rend. Acad. d. sc. **199**:618, 1934.

89. Harde, E., and Philippe, M.: Compt. rend. Acad. d. sc. **199**:738, 1934.

subcutaneously 10 mg. of vitamin C for three days and then 1 M. L. D. of toxin. All died. Each of 4 guinea-pigs was given 1 M. L. D. of toxin and then injections of vitamin C. They lost weight, but 2 survived, and 4 controls died. From five to six weeks later, the survivors were readily killed by the same amount of toxin, indicating that no immunity had been established. The investigators observed no deleterious effects of vitamin C on antitoxic potency or the potency of toxin-antitoxin mixtures in vitro.

They incubated 150 mg. of vitamin C and 500 M. L. D. of toxin at p_H 4 to 4.4 at room temperature for eighteen hours. The toxin was definitely inactivated, and from 1½ to 2 M. L. D. were no longer fatal.

They concluded that vitamin C under certain conditions increases the resistance of guinea-pigs to 1 M. L. D. of a standardized diphtheria toxin, and that diphtheria toxin kept in contact with vitamin C for one hour before injection is less toxic.

This is consistent with the observations of Lyman and King,[90] who noted a marked loss of vitamin C in the adrenal glands, pancreas and kidneys of guinea-pigs that had received injections of diphtheria toxin. In 39 animals given toxin, the decrease in vitamin C in these organs varied from 21 to 38 per cent as compared with 39 controls given similar injections. The concentration of vitamin C was estimated by titration with 2,6-dichlorophenolindophenol. Apparently, the physiologic need of vitamin C in guinea-pigs given diphtheria toxin is greatly increased.

The observation that guinea-pigs having a high storage of vitamin C are less sensitive to diphtheria toxin than are those having a low storage of this vitamin may be important in relation to the pathogenesis of diphtheria infection.

Kligler[91] attempted to ascertain the effect of vitamin C on the production of toxin by Corynebacterium diphtheriae in vitro. He studied the influence of the vitamin in the culture medium on the toxicity of filtrates of cultures of a toxin-producing strain. A sterile solution of vitamin C was added to the culture medium in concentrations ranging from 0.05 to 1.25 per cent. The flasks containing the vitamin, as well as corresponding controls, were inoculated with C. diphtheriae and incubated for forty-eight hours at 30 C. The toxicity of the filtrates was determined by intracutaneous injections into guinea-pigs.

The vitamin C added to the culture medium rapidly disappeared on incubation at from 30 to 37 C. When the medium was inoculated with C. diphtheriae immediately after the vitamin was added, the vitamin disappeared more slowly. Even small residual amounts of the vitamin (from 20 to 40 mg. per hundred cubic centimeters) are sufficient to

90. Lyman, C. M., and King, C. G.: J. Pharmacol. & Exper. Therap. **56**:209, 1936.

91. Kligler, I. J.: Nature, London **138**:291, 1936.

inhibit production of toxin. The same strain of C. diphtheriae was found to produce a potent toxin in the flask without vitamin C and little or no toxin in the one containing the same medium plus cevitamic acid in the concentration of 0.05 per cent.

Kligler [91] suggested that the varying toxic character of a diphtheritic infection may depend as much on the tissue saturation with vitamin C as on the toxigenic properties of the infecting strain. He further suggested that the mildness of diphtheritic infection in tropical and subtropical countries is in some way related to the quantitative differences in the concentration of this vitamin.

Bamberger and Wendt [92] observed some beneficial effect in severe diphtheria with circulatory disturbances from the combined use of adrenal cortical hormone and vitamin C. The use of vitamin C alone they found to be without effect. Of 8 patients treated with combined adrenal cortical hormone and cevitamic acid, 5 seemed definitely improved.

The clinical use of large quantities of cevitamic acid as a supplement to specific serum in the treatment of diphtheria has yielded unencouraging results. Otto [93] stated that the cevitamic acid at best tends to modify to some degree the hemorrhagic tendency, particularly when thrombopenia is present.

He [93] treated a series of 50 patients with diphtheritic antitoxin alone and compared the results with those obtained in 42 patients treated with antitoxin and cevitamic acid. In the patients treated with serum alone the membrane disappeared in an average period of six days and the fever dropped to normal in an average period of 7.5 days. Practically identical with these results were those in the group given large doses of cevitamic acid in addition to the antitoxin. The number of patients with severe complications was the same in both groups, and 2 patients in each group died. The cevitamic acid was given intravenously, in amounts as high as 500 mg. a day, and also as a 10 per cent spray directly to the membranous areas.

Anaphylactic Shock.—Vitamin C may have a protective effect against anaphylactic shock in guinea-pigs, which is more marked when the vitamin C has been administered prior to sensitization than when it has been given after sensitization (Salomonica [94]).

Salomonica [94] utilized 91 guinea-pigs. Each was sensitized by an intra-abdominal injection of 1 cc. of a 1:100 dilution of horse serum and shocked twenty-one days later by an intravenous injection of 0.5 cc. of a 1:10 dilution of horse serum. This amount had been found to kill the sensitized animal. In all, eight groups of animals were used. One group of 17 animals was sensitized and shocked and received no treatment with vitamin C. All succumbed. In a

92. Bamberger, P., and Wendt, L.: Klin. Wchnschr. **14**:846, 1935.
93. Otto, H.: Klin. Wchnschr. **15**:1510, 1936.
94. Salomonica, B.: J. Immunol. **31**:209, 1936.

second group of 9, the guinea-pigs received crystalline vitamin C in amounts varying from 1 to 10 mg. a day during a period of three weeks prior to and after administration of the sensitizing dose. All of these survived. The third group received vitamin C in the same amounts during a period of three weeks prior to sensitization; of 17 guinea-pigs, 14 survived. In a fourth group the vitamin C was given during three weeks after the sensitizing dose; of 16 guinea-pigs, 4 survived. In a fifth group the vitamin C was administered only during the last two weeks of the period of sensitization, and of 9 animals, none survived. In a sixth group 11 animals received the vitamin C during the last week of the period of sensitization and, of these animals, 3 survived. In a seventh group the vitamin C was given in a single dose from one to four hours prior to the injection of the shocking dose; of 7 guinea-pigs, 1 survived. Finally, the cevitamic acid was administered simultaneously with the shocking dose of horse serum, and 2 of 3 guinea-pigs were protected when 10 mg. of the vitamin C was used. In all cases, a crystalline product of vitamin C diluted in distilled water was employed.

The results support the conclusion that vitamin C administered to guinea-pigs prior to sensitization protects such animals against anaphylactic shock. The administration of vitamin C during sensitization has much less effect. When the vitamin is administered with the shocking dose, some protective value is observed. Salomonica [94] suggested that the protective action of vitamin C against anaphylaxis is dependent on its effect on the endocrine glands. He argued that hyperfunction of the thyroid gland increases and diminished function tends to decrease allergic phenomena. In guinea-pigs removal of the thyroid gland had a protective effect against anaphylactic shock, according to Lanzenberg and Képinow [95] and Kreitmair,[96] who observed that the lethal effect of thyroxine can be completely checked by cevitamic acid. This antagonistic effect of vitamin C might explain in part, he believed, its protective action against anaphylactic shock. That cevitamic acid tends to depress hyperactivity of the thyroid gland is suggested by the work of Marine and Baumann.[97]

Tuberculin Shock.—In a recent communication Steinbach and Klein [98] reported the effect of crystalline vitamin C on the tolerance of tuberculous guinea-pigs to tuberculin. In preliminary experiments they observed that cevitamic acid mixed with skin test doses of tuberculin in vitro prior to intracutaneous inoculation of those doses in tuberculous guinea-pigs failed to inactivate the tuberculin. Prolonged treatment of 7 tuberculous guinea-pigs with cevitamic acid prior to

95. Lanzenberg, A., and Képinow, L.: Compt. rend. Soc. de biol. **86**:204 and 906, 1922.

96. Kreitmair, H.: Arch. f. exper. Path. u. Pharmakol. **176**:326, 1934.

97. Marine, D.; Baumann, E. J., and Rosen, S. H.: Proc. Soc. Exper. Biol. & Med. **31**:870, 1934.

98. Steinbach, M. M., and Klein, S. J.: Proc. Soc. Exper. Biol. & Med. **35**: 151, 1936.

infection with tubercle bacilli failed to reduce the reactivity of the skin to tuberculin. They further observed that the vitamin was unable to inactivate tuberculin either in vitro or in vivo when the tuberculin was used in doses large enough to cause death of control tuberculous guinea-pigs. In the tests in vivo, the vitamin C was injected intravenously just prior to the injection of tuberculin.

They did observe, however, increased tolerance to tuberculin in tuberculous animals treated for prolonged periods with injections of cevitamic acid.

In two individual experiments 22 animals were used. Each was given a subcutaneous injection of 0.001 mg. of a bovine strain of the tubercle bacillus (B_1). From the eighteenth day after the injection of the bacilli, half the animals each received a subcutaneous injection of 5 mg. of cevitamic acid daily during the entire experimental period. Twenty-three days after infection, each of the guinea-pigs received 2 injections of old tuberculin weekly in increasing amounts of from 50 to 1,600 mg. administered subcutaneously. Whenever an animal of one group died, a guinea-pig of the other group was killed for the purpose of comparing the extent of the disease. After the eighteenth injection of tuberculin, all the untreated animals died of tuberculin shock, and only one of the vitamin-treated group.

They found that practically every one of the animals given cevitamic acid survived injection of an amount of tuberculin which killed the untreated tuberculous guinea-pig. They also noted that the untreated animals showed more extensive tuberculosis than did the animals treated with cevitamic acid. They further compared the cortices of the adrenal glands of the animals that died of tuberculin shock with those of the treated surviving guinea-pigs, by means of the silver nitrate reaction. There was no reduction of the silver nitrate (i. e., no cevitamic acid) in the adrenal glands of the shocked animals, but some was present in those treated animals which survived the shock.

They concluded that an excess of cevitamic acid given to tuberculous animals decreased their sensitivity to tuberculin shock. It may be mentioned that the cevitamic acid in the adrenal cortex of the animal dying of an infection or of a debilitating process is rapidly exhausted in the processes leading to death. The reaction is further dependent on the presence of a peroxidase enzyme, which is likewise destroyed with the death of the animal. The comparison of this reaction in animals that die of tuberculin shock with that in animals killed for control observations may not be valid.

It will be recalled that Sulzberger and Oser [15] observed hypersensitiveness to neoarsphenamine in guinea-pigs given less than 0.2 mg. of cevitamic acid per day, but when the animals were given from 1 to 5 mg. a day their capacity for allergic response to neoarsphenamine

disappeared. The investigators concluded justifiably that this hypersensitiveness was due to a relative state of insufficiency of vitamin C. This suggests that certain experiments on the effect of what is interpreted as an excess of vitamin C on resistance to toxins, infection and anaphylactic shock may be open to criticism. It is possible that the control animals employed in such studies may really have been suffering from a relative insufficiency of vitamin C at the time of the onset of the experiment, and that the experimental animals that received additions of vitamin C in the diet represented those fed a diet physiologically adequate in vitamin C and not containing necessarily an excess of the vitamin.

Poliomyelitis.—Jungeblut [99] observed that substances neutralizing the virus of poliomyelitis were present in serum, human tears, placenta, the urine of pregnant women, extracts of adrenal gland, extract of adrenal cortex, epinephrine and serum containing diphtheritic antitoxin. He then studied the effect of vitamin C in neutralizing the virus of poliomyelitis.

To portions of the supernatant fluid of a 10 per cent emulsion of spinal cord from a monkey infected with poliomyelitis he added graded amounts of a solution of crystalline vitamin C varying from 0.05 to 50 mg. Then 0.1 cc. quantities of these mixtures were injected into 30 monkeys, respectively. Inactivation was obtained with 1, 5, 10 and 50 mg. Amounts smaller than 0.1 and larger than 10 mg. were not constantly effective, and 50 and 100 mg. were toxic.

He concluded that multiple paralytic doses of the virus of poliomyelitis when mixed with very small amounts of crystalline vitamin C are rendered noninfectious, as determined by intracerebral injection of such mixtures into monkeys (Macacus rhesus).

Recently Jungeblut [100a] reported before the American Bacteriological Society experiments in vivo on the action of vitamin C in preventing poliomyelitis in monkeys. By injecting small doses of vitamin C subcutaneously in monkeys, beginning with the day on which the animals were inoculated with the virus, he found that the treated animals were more resistant than the controls.

He divided 30 monkeys into four groups. Three groups were given subcutaneous injections of varying amounts of crystalline vitamin C from the day of injection daily for two and a half weeks. The fourth group was left untreated, as controls. The monkeys treated with 5 mg. of vitamin C survived without showing any signs of poliomyelitis. In 10 monkeys which had had no treatment with vitamin C but which had received the same dose of virus, poliomyelitis developed.

99. Jungeblut, C. W.: J. Exper. Med. **62**:517, 1935.

100. Jungeblut, C. W.: (*a*) J. Bact. **31**:34, 1936; (*b*) J. Exper. Med. **65**: 127, 1937.

Subsequently Jungeblut [100b] extended these experiments and confirmed his earlier results.

A group of 34 rhesus monkeys, weighing between 2,000 and 3,000 Gm. each, were inoculated intracerebrally with 0.1 cc. of a 10 per cent suspension of the virus. Following the inoculation, 9 animals were treated with daily injections of from 700 to 100 mg. each of crystalline natural vitamin C, 16 with from 50 to 10 mg. and 9 with 5 mg., during a period of two weeks—34 tested monkeys in all. Six of the treated monkeys survived without showing paralysis; 2 of these had received from 50 to 10 mg., while 4 had received 5 mg. Of 19 untreated monkeys simultaneously infected with the same amount of virus, all showed paralysis.

In a second group, 13 monkeys were given intracerebral injections of 0.05 cc. of the virus. Following the injection, 1 animal was treated with 25 mg. and 5 animals with 5 mg. of vitamin C daily for a period of two weeks; 7 were untreated. One animal that had received 5 mg. daily survived without evidence of paralysis. Of the untreated monkeys, all presented paralysis.

In a third experiment, 22 monkeys were infected intracerebrally with 0.01 cc. of the virus. Of this group, 1 was treated with 100 mg., 2 with 50 to 10 mg. and 19 with 5 mg. of vitamin C daily during a period of two weeks. Of this group 1 that received 10 mg. and 11 that had received 5 mg. survived without showing paralysis. Of 12 untreated control monkeys similarly infected with the same dose, 2 failed to show paralysis, and 10 subsequently had paralysis.

In summarizing his results, Jungeblut [100b] stated that of a total of 62 treated monkeys, 19 survived without paralysis; 43 succumbed to the disease. Of a total of 38 untreated controls, only 2 failed to succumb to paralysis. All monkeys treated with vitamin C in doses of from 700 to 100 mg. presented paralysis; 3 of 19 monkeys that received 50 to 10 mg. survived without paralysis, while nearly half of the animals receiving 5 mg. escaped this disease.

The use of vitamin C prophylactically against subsequent intracerebral infection failed to protect monkeys in any instance. Of 2 control animals that were given crystalline carotene in amounts of 100 mg. for two weeks and two that received 2 Gm. of vitamin B in the form of powdered yeast, following injection of the virus, all succumbed with paralysis. The addition of vitamin C directly to normal nonneutralizing monkey serum gave irregular results.

Jungeblut [100b] is of the belief that the susceptibility of certain children and adults to the virus of poliomyelitis is dependent in part on a partial deficiency of vitamin C in the diet. The majority of children and most adults fail to contract poliomyelitis when exposed to the infectious agent. In some instances, this is due to immunity acquired in a paralyzing attack, and this immunity may or may not be associated with circulating antibodies. The more important type of protection is represented by natural resistance to the disease. It is believed by some epidemiologists that the widespread resistance to the virus is due to the immunizing effect of subclinical or abortive attacks of the disease. Subclinical immunization in monkey and man, however, has not been

demonstrated, and the viricidal substances that are found in normal human and animal tissues, serum or other body fluids are probably not of an antibody character, according to Jungeblut, but resemble agents of a vitamin-like or hormonal nature.

Since immunization by means of killed virus had proved inadequate and that by means of live virus dangerous, Jungeblut suggested that prophylaxis and treatment of infantile paralysis must depend on enhancing the natural resistance of the host. From his results, he concluded that this might be accomplished by supplying the exposed or infected persons with optimal amounts of vitamin C.

It is known that the minimum requirement of an adult guinea-pig in health is from 1 to 2 mg. of cevitamic acid per day and that the need of vitamin C in the presence of infection is considerably greater.[101] Five milligrams of vitamin C daily, the dosage utilized in many of the experiments reported in the literature, may not provide an excess of this substance.

The problem of the effect of an excess of vitamin C on the natural resistance of man and animals, such as the guinea-pig or the monkey, requires further investigation. Animals utilized in such experiments should be of a stock known to be constant, one maintained under controlled conditions, on an adequate standard diet containing cevitamic acid in a physiologically adequate amount from the day of birth. Comparisons of the resistance of such animals to infection may then be made with that of animals given a sufficient excess above this minimal adequate amount for different intervals of time. In this way it can be determined to what degree the natural resistance to infection may be raised above the normal level by the administration of vitamin C.

Virus of Herpes.—Holden and Resnick[102] studied the effect of vitamin C on inactivation of the virus of herpes in experiments in vitro. They observed that cevitamic acid failed to inactivate "w" virus, which is immunologically related to the virus of herpes, when the p_H of the virus-vitamin mixture fell within the rnage of p_H 5 to p_H 8. When p_H was less than 5, the cevitamic acid inactivated the virus. It was apparent that the action of the cevitamic acid on the "w" virus is nonspecific and due entirely to the effect of p_H.

Trypanosoma Equiperdum.—An excess of vitamin C had no influence in modifying the course of infection with T. equiperdum in mice (Perla[103]).

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101. It is further known that animals fed a relatively inadequate amount of vitamin C and then given in addition an infection may be thrown into a more advanced state of vitamin C insufficiency and even frank scurvy.

102 Holden, M., and Resnick, R.: J. Immunol. **31**:455, 1936.

103. Perla, D.: To be published.

The mice were on a complete and adequate diet, consisting of rolled oats, hominy, meat scrap, dried milk and salt, with daily additions of fresh milk, yeast, cod liver oil and wheat germ. Fresh greens were added twice a week. Mice thrive and gain rapidly on such a diet. The mice had been bred from a strain originally of the Rockefeller Institute for Medical Research and were free from latent infections. The strain of T. equiperdum had been carried in guinea-pigs for several passages to separate it from Bartonella muris, as this organism is destroyed in the guinea-pig. The mice were given daily intraperitoneal injections of a solution of synthetic cevitamic acid in amounts of 1 mg. per day per mouse during a period of fourteen days prior to the injection of trypanosomes and thereafter. The minimal effective dose of trypanosomes necessary to produce an infection in the normal animal had been determined in preliminary experiments. From ten to thirty times this amount was used to insure uniform "takes." The experiments with vitamin C were repeated three times on different occasions, an equal number of controls being used in each experiment. The course of the infection and the duration of life were studied in all the experiments.

No modification of the infection was observed in any of the experiments as a result of the injections of cevitamic acid. The resistance of the mice was not modified by the available excess of cevitamic acid.

These experiments, though suggesting that the resistance of mice to a protozoan infection cannot be altered by an excess of vitamin C parenterally administered, were made in a species known to produce its own vitamin C. It is well established that scurvy cannot be established in rats or mice on diets deprived completely of vitamin C during as long a period as three generations (Simmonds [104]).[105] It was felt that since rats and mice are capable of producing their own vitamin C the results of studies of the effect of an excess of this factor on their resistance could not be interpreted.

In another series of experiments, therefore, Perla [103] studied the effect of repeated injections of cevitamic acid on trypanosomal infection in guinea-pigs.

The guinea-pigs were of a uniform stock, which had been raised in the laboratory for more than ten years, on a uniform adequate diet of oats, alfalfa hay, greens ad libitum and water. Cevitamic acid was injected daily, intraperitoneally, in amounts of 10 mg. dissolved in physiologic solution of sodium chloride, brought up to a p_H of 7.5, during a period of forty-two days prior to the injection of trypanosomes and thereafter until the death of the animal. In the first experiment, 12 guinea-pigs were treated with cevitamic acid, and 12 were con-

104. Simmonds, N.: Am. J. Hyg. (supp.) **4**:1, 1924.

105. No difference in rate of growth or reproductive capacity is observed in rats fed a diet deficient in vitamin C. G. A. Hartwell (Biochem. J. **24**:967, 1930), H. T. Parsons (J. Biochem. **44**:587, 1920), and S. Lepkovsky and M. T. Nelson (J. Biol. Chem. **59**:91, 1924) found the livers of rats fed a scurvy-producing diet rich in antiscorbutic substance. The livers of rats of a second generation raised on a diet free from vitamin C were still equally rich in vitamin C. Rats fed a diet deficient in vitamin C showed no decrease in their capacity to produce immune antibodies.[103]

trols of the same age (from 6 to 7 weeks). Each animal received 30,000 trypanosomes intraperitoneally. Of the treated guinea-pigs, only 2 became infected; they died within twenty-five and thirty days. Ten escaped infection. Of the untreated ones, all succumbed.

In a second experiment, the same daily quantities of vitamin C were given during a period of seventy-seven days prior to the injection of T. Brucei. The infecting dose was 30,000 trypanosomes. Of the 7 guinea-pigs treated, all succumbed to the infection within a period of from eleven to twenty-seven days (average, nineteen days). Of the 7 controls, 6 succumbed and 1 survived. The duration of the infection varied from nineteen to thirty-one days (average, twenty-six days). The interval between the injection of the trypanosomes and the appearance of organisms in the blood stream was nine days in the control and from five to seven days in the vitamin C-treated guinea-pigs. The treated animals lost considerably in weight. These animals were from 4 to 5 months of age. The difference in the two groups is not significant.

In the third experiment, still in progress, the conditions were more carefully controlled. A group of 24 guinea-pigs was removed from stock at the age of 5 weeks, and each animal was given 1 mg. of cevitamic acid daily intraperitoneally. After a short period, 12 of the guinea-pigs received 10 mg. daily, and the others, as controls, continued to receive 1 mg. daily. The diet was adequate as in the previous experiments.

At the end of forty days of treatment each animal was given an injection of 50,000 trypanosomes (Trypanosoma Brucei), and the injections of vitamin C were continued. (Use of individual syringes and needles in administration of vitamin C after infection is important, to avoid cross-infections.) A manifest infection developed in all animals. After a period of six weeks 10 of the controls had died, whereas only 2 of the 12 that had received 10 mg. a day had died. The rest, at the time of writing, either showed remission or had a very mild infection.

It was thought that this type of experiment eliminated the objections as to the uncertainty of the dosage of vitamin C in the controls. The protective effect of an excess of vitamin C on resistance was more pronounced in the young animals.

In moderate excess cevitamic acid raises the natural resistance of the guinea-pig to a trypanosomal infection.

Summary.—A moderate excess of cevitamic acid may increase the natural resistance of guinea-pigs to diphtheria toxin and tuberculin and to anaphylactic shock. Both diphtheritic toxin and the virus of poliomyelitis may be neutralized by this acid in vitro. A moderate excess of vitamin C, parenterally introduced, raises the resistance of monkeys to the virus of poliomyelitis and of guinea-pigs to T. Brucei. Large quantities of cevitamic acid may be toxic to guinea-pigs.

The literature on the effect of an excess of vitamin C containing foods on human infections such as tuberculosis is of little worth. It is suggested, though it is not as yet well established, that an excess of vitamin C raises the resistance of animals to intestinal tuberculosis. Therapeutically, in tuberculous animals a great excess of vitamin C administered for long periods is apparently of little value in modifying the severity of the general disease.

X. IMPORTANCE OF VITAMIN C IN MAINTENANCE OF NORMAL TOOTH GROWTH

The importance of the carious tooth as a portal of entry for bacterial infection has been well established. In recent years the rôle of dietary factors in the maintenance of normal tooth structure and growth has been stressed. The earliest and most characteristic changes caused by vitamin C deficiency are found in the teeth and gums (Dalldorf and Zall,[106] Höjer and Westin,[107] Wolbach and Howe [108]).

Zilva and Wells [109] were the first to stress the fact that scurvy even in the mildest form produces marked changes in the structure of the teeth. Fibroid degeneration takes place in the tooth pulp. When the fibrosis is complete, no cellular elements, no trace of cellular organization, no cement substance is seen. The nerve cells, blood vessels and odontoblasts all share in the fibroid degeneration and are no longer recognizable. The irregular osteoid condition of the dentin is well marked, and the refractive appearance of the dentin is due to a hemorrhagic condition of dentinal fibrils. The change starts first in the odontoblastic cells at the top of the pulp, working down toward the apex, and is followed by engorgement of blood vessels and hemorrhage, leading to complete fibroid degeneration.

The teeth are affected as early as the seventh day in guinea-pigs placed on a diet of oats, bran and autoclaved milk. When scorbutic conditions during life are so slight as to be almost unrecognizable the condition of the teeth may be severe. The hemorrhagic phenomena in muscle and periosteum and the changes at the epiphyses of the long bones and ribs appear at a much later period.

Robb and his co-workers [110] studied the pathogenesis of the changes in the pulp in greater detail. In the normal guinea-pig, the odontoblastic layer of cells is of the tall columnar type, and the inner surface of the dentin is smooth. The pulp contains a fine fibrillar connective tissue stroma and is well supplied with blood vessels. In scurvy, the odontoblasts lose their columnar form, and some of them are enclosed in osteodentin. The odontoblasts secrete osteodentin rapidly, and the osteodentin soon completely encloses the pulp cavity. It is thick, and the inner surface is thrown into folds; there is hyperemia of the pulp, and small hemorrhages are present. There is increased elimination of

106. Dalldorf, G., and Zall, C.: J. Exper. Med. **52**:57, 1930.

107. Höjer, A., and Westin, G.: Dental Cosmos **67**:1, 1925.

108. Wolbach, S. B., and Howe, P. R.: Proc. Soc. Exper. Biol. & Med. **22**:400, 1924. Wolbach and Howe.[31]

109. Zilva, S. S., and Wells, F. M.: Proc. Roy. Soc., London, s.B **90**:505, 1919.

110. Robb, E. F.; Medes, G.; McClendon, J. F.; Graham, M., and Murphy, I. J.: J. Dent. Research **3**:39, 1921.

calcium in the scorbutic state, and the teeth rapidly lose calcium and cementin. The osteodentin eventually fills the pulp cavity. Robb and his co-workers [110] estimated that in scurvy the osteodentin of the pulp cavity is a hundred times greater than the normal. The foregoing observations are not entirely accurate.

The changes in the jawbones and teeth were further investigated by Höjer and Westin [107] and the reconstruction of the pathogenesis modified:

In the jawbone, the calcified bone becomes porous through a process which starts in the marrow spaces and the bone canals. New bone is formed on the outer surfaces of the jawbone. This bone is deficient in collagen and inferior in quality.

In the teeth, the first positive sign of scurvy, according to Höjer and Westin,[107] is a gradual change and disappearance of the odontoblastic layer. There is amorphous calcification of the predentin, with disappearance of Tomes' canals in this layer. The dentin that was calcified at the onset of the scurvy becomes porous through a process which starts from the protoplasm content of Tomes' canals and results in their dilatation and confluence. A new formation of pulp bone instead of dentin occurs. This bone has a spongy structure and an appearance distinctly corresponding with that of the new-formed, inferior bone of the outer surfaces of the jawbone. Dilatation of the pulpal vessels and hemorrhages occur. Subsequently, hydropic degeneration of the pulp develops with necrosis. Finally, after new formation of bone in the pulp has stopped, atrophy and resorption of this bone, of the dentin and of the pulp occur. Large cystic areas filled with fluid may replace the disappearing pulp.

In latent scurvy, the process is similar but less pronounced. If an insufficient quantity of antiscorbutic substances is given in the diet, irregular dentin is formed, which has Tomes' canals. In the lingual part of the pulp there are symmetrically arranged ridges of pulp bone without dental canals.

Höjer and Westin [107] observed an overproduction of osteodentin such as was described by Robb and his associates [106] in those instances in which as much as 80 per cent of the minimal protective quantities of vitamin C was given in the diet. In such instances, no pulp bone was laid down, all the newly formed hard tissue consisting of osteodentin.

Wolbach and Howe,[108] in a careful review of the pathologic changes in the teeth in complete scurvy, offered interpretations differing in some respects from those of Höjer and Westin.[107] They observed that in complete scurvy in guinea-pigs there is no formation of osteodentin or pulp bone, but that these represent regenerative processes due to the presence of small amounts of antiscorbutic substance. The earliest

changes noted when all vitamin C is withdrawn from the diet occur in from seven to twelve days as a separation of the odontoblast layer from the dentin by a narrow margin, occasional irregular calcium deposits in the odontogenic zone (predentin) and irregularities in the odontoblast layer. The individual cells become smaller and stain more deeply. The blood vessels are engorged. In the predentin zone there are occasional deposits of basic-staining granular material, interpreted as calcium salts, while between the processes of the odontoblasts continued formation of dentin can be inferred because of the presence of globules of hyaline material interpreted as a matrix of spherites. After from twelve to fourteen days, complete separation of the odontoblasts from the dentin occurs. There is rupture of the processes of the odontoblasts, and the spaces separating the odontoblasts and the dentin contain no stainable material. Such spaces appear first as vacuoles, and it is probable that an accumulation of liquid material causes the separation. The odontoblasts exhibit changes in size and increased density of staining. Changes in the pulp occur as edema and granular deposits between the connective tissue. In absolute scurvy, the pulp is shrunken, freed from dentin and apparently floating in a liquid material. Within twenty-four hours after the administration of orange juice or vitamin C a zone of new dentin forms on the separated odontoblasts, and within three days of treatment the new dentin completely fills the space between the odontoblast layer and the dentin layer. It appears irregular in contour, following the irregular surface of the odontoblasts. This Wolbach and Howe [108] interpreted as a jelling of the defective material. They hypothecated that in scurvy the odontoblasts continue to produce a liquid material. This theory accounts for the edematous appearance of the fibrous tissue-like structure in bones called *Gerüstmark*. The theory is supported by the promptness and the volume of the matrix formation following antiscorbutic treatment.

These observations have been confirmed in detail by Hanke,[111] who in a recent monograph pictorially reviewed the changes in progressive scurvy. In addition, he studied the reparative process in guinea-pigs fed a scorbutic diet for twenty-one days and then given vitamin C as orange juice in amounts of 5 cc. a day for twenty days. Essentially, the reparative process involved partial restoration to the normal state. With additions of vitamin C, a network of calcified organic matter appeared in the pulp. Calcification was most pronounced in the regions contiguous to the original dentin. Spicules of calcified organic matter radiated toward the center of the pulp. At two days, odontoblasts were not in evidence, and the pulp was still very abnormal, containing many vacuoles and no normal pulp cells. At five days, the radiating network of

111. Hanke, M. T.: J. Am. Dent. A. **17**:957, 1930.

calcified organic matter had advanced from all sides almost to the center of the pulp chamber. Cells resembling odontoblasts were seen growing over all the surfaces of the abnormal calcific deposits. At ten days, the pulp chamber had become almost a solid mass of calcific organic matter. At twenty days, the calcified organic matter in the pulp chamber had acquired an appearance similar to that of imperfectly formed dentin. Odontoblasts appeared at the apical end, and tubules were apparent in the imperfectly formed dentin at this end.

In those instances in which the diet had contained vitamin C in small but insufficient amounts for twenty-one days, the addition of adequate amounts of vitamin C was followed by a restoration of the tooth structure practically to the normal. Thus, after five days of treatment, odontoblasts appeared in large numbers and distributed themselves along the inner margins of the imperfectly formed dentin. New tubules appeared in the imperfectly formed dentin contiguous to the odontoblasts, and the pulp was firmer and more fibrotic in structure. After ten days of treatment, the newly formed tubules extended to the zone where the original predentin had become amorphously calcified with the onset of scurvy. The amorphously calcified predentin that marked the region of the original injury was vague, but the pulp still exhibited many wide spaces, about some of which the odontoblasts were distorted. Between the spaces, the cells of the pulp appeared to be normal.

The observations on the changes in the teeth in scurvy indicate the interesting fact that even with subclinical scurvy the transformation of the pulp may go beyond the point where complete restoration to normal can occur, regardless of treatment. That is, some phase of the injury leaves permanent distortion. In milder scurvy the restoration may proceed further but apparently is not complete.

Hanke[111] believed that the incidence of dental caries could be checked by a diet rich in vitamin C, but this has not been established either clinically or experimentally.

There is some evidence that gingivitis may be perceptibly improved by an excess of vitamin C, for it is likely that this condition indicates relative absence of vitamin C.

It is established that vitamin C is important in normal tooth growth and tooth structure and in the maintenance of a healthy condition of the gums. Indirectly, therefore, it must be important in the natural resistance of the pulp and peridental tissue to focal infection.

XI. INTERPRETATION OF THE RÔLE OF VITAMIN C IN RESISTANCE

Physiologic Importance of Vitamin C in Formation of Intercellular Cement.—Vitamin C is physiologically important in oxidation-reduction systems in the body. Its properties were described chemically by Szent-Györgyi,[2] who isolated a reducing substance from the adrenal cortex

and found an identical principle in cabbage and orange juice. His interest was directed to the substance in the course of a study of peroxidase systems in plants. The reducing principle that he isolated he believed to be a hexuronic acid and later demonstrated its anti-scorbutic properties and its identity with vitamin C.[8] The structure was subsequently found to be a diketo-acid showing tautomeric change (Haworth, Hirst and Reynolds,[112]) and its synthesis from xylosone was described by Reichenstein, Grussner and Oppenauer.[113] For a complete review of the recent advances in the chemistry of cevitamic acid, the reader is referred to the excellent survey by King.[114]

A deficiency of vitamin C leads to a change in the intercellular cement substance (Wolbach and Howe [108]). Through the careful studies of Aschoff and Koch,[115] Höjer and Westin,[107] and finally Wolbach and Howe, the essential effects have been established. Wolbach and Howe have contributed the most plausible explanation of the changes seen in vitamin C deficiency from their excellent work on guinea-pigs. They observed the sequence of events in the absolute scorbutic state in the incisor teeth and the bones of growing guinea-pigs and they studied the process of repair of the soft tissues and bone following injury in scorbutic guinea-pigs. In scurvy, there is an inability of the supporting structures to produce and maintain intercellular substances. Direct proof of this conclusion has been obtained in the study of teeth in regard to the dentin, in the study of growth and repair of bone in regard to the bone matrix and in the study of repair of soft tissue in regard to the collagen of connective tissues.

The failure of capillary formation is due to the failure of endothelial cells to form cement substances. The proliferative power of epidermis, endothelium, fibroblasts and osteoblasts is not diminished in scorbutus. The osteoblasts, in spite of morphologic change, preserve their chemical potentialities and produce a bone matrix. The odontoblasts continue to produce a liquid material which lacks jelling properties. A failure of the cement substance in blood vessels accounts for the hemorrhage. This failure of the cell to produce intercellular substance in scurvy is due to absence of an agent common to all supporting tissues which is responsible for the setting or jelling of a liquid product.

This explanation helps to clarify much of the pathologic picture. It is possible that changes in the resistance of scorbutic animals to infection

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112. Haworth, W. N.; Hirst, E. L., and Reynolds, R. J. W.: J. Soc. Chem. Indust. **52**:482, 1933.

113. Reichenstein, T.; Grussner, A., and Oppenauer, R.: Helvet. chim. acta **17**: 570, 1934.

114. King, C. D.: Physiol. Rev. **16**:238, 1936.

115. Aschoff, L., and Koch, W.: Veröffentl. a. d. Geb. d. Kreigs- u. Konstitutionspath. **1**:4, 1919.

are in part attributable to the altered permeability of membranes resulting from this change. That is, there is a breakdown in the normal protective capacity at the site of bacterial invasion of the body. This also suggests a decrease in the resistance of the tissue in general to injury of any type.

Physiologic Importance of Vitamin C in Cellular Respiration.—With the recent advance in knowledge of the chemistry of vitamin C, it becomes apparent that this vitamin is essential in reduction-oxidation processes in all cells of the body. Though existing in higher concentration within the adrenal cortex, it is found in the ovaries, brain, liver (Bessey and King [116]), pituitary gland, pancreas and kidney, and large amounts are present in the thymus (von Euler and Klussmann [117]). Its presence in association with cholesterol or glutathione may be of significance. Since the adrenal gland is a tissue of extremely high metabolic activity, it would of necessity utilize in its physiologic processes large amounts of so important a reducing substance as cevitamic acid. The utilization of the vitamin elsewhere in the body or the capacity of certain animals to manufacture it may not be dependent specifically on the adrenal cortex. The possibility of a specific relationship was suggested by the fact that vitamin C and the adrenal hormones are concerned with oxidation-reduction systems in animal metabolism, that adrenalectomized animals present hemorrhagic phenomena suggestive of those in scorbutic animals, and that cevitamic acid occurs in large amounts in the cortex. A specific interrelationship has not, however, been demonstrated.

Svirbely and Kendall [118] studied the influence of cortical hormone on the nitrogen metabolism of adrenalectomized dogs maintained on a vitamin C-free diet. No changes in nitrogen metabolism occurred under these circumstances. In the absence of the adrenal cortical hormone in adrenalectomized animals, the nitrogen metabolism tends to become negative (Sandberg and Perla [119]). Svirbely and Kendall observed no changes suggestive of scurvy in adrenalectomized dogs maintained on a vitamin C-free diet but given extract of adrenal cortex. No mention is made of a detailed histologic examination of bones.

Nevertheless, Lockwood and Hartman [120] and Lockwood, Swan and Hartman [121] have observed that an extract of adrenal cortex relatively

116. Bessey, O. A., and King, C. G.: J. Biol. Chem. **103**:687, 1933.

117. von Euler, H., and Klussmann, E.: Ztschr. f. physiol. Chem. **217**:167, 1933.

118. Svirbely, L. J., and Kendall, E. C.: Am. J. Physiol. **116**:187, 1936.

119. Sandberg, M., and Perla, D.: J. Biol. Chem. **113**:35, 1936.

120. Lockwood, J. E., and Hartman, F. A.: Endocrinology **17**:501, 1933.

121. Lockwood, J. E.; Swan, D. R., and Hartman, F. A.: Am. J. Physiol. **117**:553, 1936.

free from vitamin C ameliorated the symptoms of scurvy. Control extracts of liver offered no protection. They noted that a definite amount of cortical extract afforded optimum protection, and greater amounts did not increase the effect. They expressed the belief that the substance is not vitamin C, present as a contaminant, but that cortical hormone or some unknown substance from the adrenal cortex delays the onset of scurvy.

In another connection, Schour and Rogoff [122] studied the histologic changes in the teeth of rats at various intervals after adrenalectomy. Slight changes were noted in the character of the predentin. They suggested that these changes are associated with a disturbance of calcium metabolism.

Perla and Sandberg [123] reported a series of experiments undertaken to determine whether an animal such as the rat, normally capable of producing its own vitamin C, shows impairment of this capacity in the absence of the adrenal glands. They observed microscopic changes in the teeth due to adrenalectomy somewhat similar to the changes noted by Schour and Rogoff.[122] These changes consisted in disappearance of the predentin zone or condensation and calcification of this zone, irregularity of the odontoblast layer, and vacuolation and pyknosis of the nuclei. Occasionally, metaplasia of the dentin with formation of islands of osteoid tissue occurred.

Adrenalectomy in the rat was found to be unassociated with a significant decrease in the vitamin C stored in the liver or in the vitamin C excreted in the urine.[124] Further, the dental changes were found in animals regardless of the vitamin C content of the food: they may not, therefore, be considered an expression of vitamin C deficiency but rather are due to a metabolic disturbance produced by the removal of the adrenal gland.

The changes in the teeth of adrenalectomized rats, though at first suggestive of early changes seen in scurvy in the guinea-pig, are apparently not an expression of an impairment of the ability of the rat either to utilize or to synthesize vitamin C, nor are they an expression of an inhibition of cellular respiration per se. This is probably true, since repeated injections of methyl cyanide, a drug that inhibits cellular oxidation, into normal rats does not produce alterations of this type. The tooth changes are not an expression of a disturbance in calcium metabolism, since chemically the disturbance in calcium metabolism after

122. Schour, I., and Rogoff, J. M.: Am. J. Physiol. **115**:334, 1936.

123. Perla, D., and Sandberg, M.: Arch. Path. **23**:372, 1937.

124. Nevertheless, they did observe that prolonged maintenance of rats not subject to operation on a scorbutic diet results in condensation and calcification of the epiphyses of the long bones, with complete cessation of growth of the cartilaginous columns. No changes in the teeth of these animals were observed.

adrenalectomy is too slight to be of significance in tooth metabolism (Sandberg and Perla[125]). It was further observed that the decrease in natural resistance to histamine poisoning following adrenalectomy in the rat cannot be influenced by injections of an excess of cevitamic acid (Perla[126]). From these observations, Perla and Sandberg[128] concluded that the adrenal gland is probably not essential in the production or the utilization of vitamin C. A specific interrelationship is not present.

From the work of Bourne[127] and Giroud, Leblond and Giroux,[128] it is probable that almost every cell in the body contains traces of vitamin C. Bourne[129] suggested that vitamin C forms an oxidation-reduction system with glutathione, and it is likely that it is important in cellular respiration. It is thus nonspecifically associated with the production of hormones in the endocrine glands.

It is now well established that vitamin C is important as an agent for the transportation of hydrogen between unidentified metabolites and other carriers by way of two or more oxidase enzyme systems. It is therefore essential in cellular respiration.

The importance of vitamin C in resistance is secondary, then, to its essential rôle in the maintenance of normal cellular metabolism. By its withdrawal, cellular respiration is promptly inhibited, and the threshold of tolerance to poisons, infections or other types of injury is necessarily depressed. It is possible that an excess of cevitamic acid may raise the threshold of tolerance, but such effect, by the nature of the chemical processes involved, would be limited in its possibilities. However, since it has been shown that latent scurvy may be converted into clinical disease in the presence of mild infection, it is apparent that the physiologic need for vitamin C must be greater in conditions in which the rate of cellular metabolism is increased. In the presence of infection, therefore, an increase in the intake of vitamin C may be of importance.

Detoxifying and Bactericidal Action of Vitamin C.—It has been suggested by the work of Harde[88] and Harde and Philippe,[89] Jungeblut and Zwemer,[86] Kligler[91] and others that vitamin C may have a direct detoxifying effect on bacterial toxins. According to Kligler[91] and Gagyi,[130] its value in the treatment of infectious diseases may be

125. Sandberg, M., and Perla, D.: J. Biol. Chem. **113**:35, 1936.
126. Perla, D.: Proc. Soc. Exper. Biol. & Med. **35**:390, 1936.
127. Bourne, G.: Nature, London **131**:874, 1933.
128. Giroud, A.; Leblond, C. P., and Giroux, M.: Compt. rend. Acad. d. sc. **198**:850, 1934.
129. Bourne, G.: Physiol. Rev. **16**:442, 1936.
130. Gagyi, J.: Klin. Wchnschr. **15**:190, 1936.

dependent on its inhibitory action on bacterial growth as well as on toxin formation.

The action of vitamin C in neutralizing diphtheria toxin in vitro may be dependent on its acidifying effect as well as on its action as a reducing agent. Its bactericidal action in vitro, according to Gagyi,[130] is due to its reducing properties. Gagyi [130] determined the bactericidal power of a 2 to 5 per cent vitamin C solution on suspensions of various microorganisms. Dilute suspensions of cultures of C. diphtheriae, C. pseudodiphtheriae, Diplococcus pneumoniae, Micrococcus catarrhalis, organisms of the typhoid-paratyphoid-colon-dysentery group and Myco. tuberculosis were placed in a 2 per cent saline solution of cevitamic acid and incubated for various periods at 37 C. The organisms were then subcultured to determine their viability, and the quantity of vitamin C destroyed in the solution was estimated by titration.

The diphtheria organisms and those of the typhoid-paratyphoid group were extremely sensitive to the effects of vitamin C and were rapidly destroyed when exposed to solutions of this vitamin. Pneumococcus and Str. haemolyticus were more sensitive and more easily destroyed than Staphylococcus. The viability of tubercle bacilli was unaffected even after seventy hours' exposure to a 5 per cent solution of vitamin C. The destruction of the vitamin C by such bacterial suspensions may be due to the formation of alkali by the bacteria as well as to the metabolic utilization of the vitamin by the bacteria (Gagyi [130]).

Whether the action of vitamin C in vivo is due either to a direct bactericidal or to a detoxifying effect is problematic. It is doubtful whether one could secure such a high degree of saturation of the cells of the body with vitamin C as is apparently necessary for bactericidal action comparable with that attained in vitro. Not only is it improbable owing to the rapidity with which vitamin C is excreted in the urine when administered in excess, but vitamin C in very large amounts would probably prove deleterious in human beings as it does in animals. It is very unlikely, therefore, that the activity of vitamin C in resistance is dependent on any direct bactericidal action. It is far more probable that it exerts its influence independently by its effect on oxidation-reduction processes in cellular metabolism.

Influence of Vitamin C on Hematopoiesis, on Growth of Monocytes and on Response to Injury.—Alterations in natural resistance in scurvy are probably independent of any interference in the production of immune antibodies or in the availability of so-called natural antibodies as opsonins, bacteriolysins and complement. However, some reduction in the phagocytic power of the leukocytes was observed with respect to Bact. coli and Myco. tuberculosis by Lawrynowicz.[131]

131. Lawrynowicz, A.: J. de physiol. et path. gén. **29**:270, 1931.

Findlay [132] observed late leukopenia in chronic scurvy in guinea-pigs and noted degenerative changes in the marrow. The leukoblastic reaction to infection is feeble in scorbutic guinea-pigs infected with pneumococci. In scurvy there is a relative increase in the number of lymphocytes, which decreases before death, according to Maxia.[133] The Arneth index is turned to the left. The reduction in the leukoblastic reaction of the organism in scurvy was observed by Lawrynowicz.[131] The intraperitoneal injection of irritating substances, such as aleuronat and peptone, into scorbutic animals called forth an exudate which was not as rich in leukocytes as that obtained in the normal guinea-pig.

Though some investigators postulate that the diminished resistance to infection in scorbutic states is secondary to these minor changes in leukoblastic response and slight alteration in opsonic power, it is probable that these are merely indications of an altered cellular activity of a more fundamental character.

Further evidence of alterations in the activity of the marrow is furnished by Mettier and Chew,[134] who observed in guinea-pigs fed a diet deficient in vitamin C a progressive profound anemia accompanied by definite cytologic changes in the marrow indicative of retarded production of red blood cells. The red cell count dropped to 2,500,000 per cubic millimeter in fifteen days, and the hemoglobin to 5.5 Gm., from a normal level of 12 Gm. per hundred cubic centimeters. The reticulocytes increased terminally to 5 and 10 per cent, from a normal of 1 per cent. The marrow of these animals showed almost complete disappearance of fat cells, marked decrease in the number of mature erythrocytes and increase in the number of normoblasts.

With the administration of orange juice, the reticulocytes rose from 12 to 20 per cent, and the anemia promptly disappeared. Sections of the marrow removed from these animals at the height of the reticulocyte response showed marked cellularity. However, in contrast to the marrow before treatment, the marrow after treatment showed marked increase in mitotic figures.

That cevitamic acid functions as one of the regulators of erythrocyte metabolism seems probable from the experiments of Baron and Baron.[135] It had been discovered by Waltner and Waltner [136] and Mascherpa [137] that cobalt salts, when given to animals, produced poly-

132. Findlay, G. M.: J. Path. & Bact. **24**:446, 1921.

133. Maxia, C.: Scritti biol. **5**:35, 1930.

134. Mettier, S. R., and Chew, W. B.: Proc. Soc. Exper. Biol. & Med. **29**: 11, 1931.

135. Baron, A. G., and Baron, E. S.: Proc. Soc. Exper. Biol. & Med. **35**: 407, 1936.

136. Waltner, K., and Waltner, K.: Klin. Wchnschr. **8**:313, 1929.

137. Mascherpa, P.: Arch. ital. de biol. **82**:112, 1930.

cythemia, as determined by the increase in the number of red cells and hemoglobin in the peripheral blood. Mascherpa [137] noted in dogs with polycythemia due to cobalt increased activity of the marrow. Baron and Baron,[135] by daily subcutaneous injection of 0.01 Gm. of cobalt sulfate, induced, within six to seven days, definite polycythemia with appearance of reticulocytes and erythroblasts in the circulating blood. The occurrence of these young cells in the blood was associated with an increase in the consumption of oxygen by the red cells to ten times that observed in controls, in which erythroblasts were not present in the circulating blood.

When cevitamic acid was injected intravenously into rabbits in amounts of 60 mg. a day simultaneously with cobalt sulfate, polycythemia failed to appear. When cevitamic acid was withdrawn, polycythemia appeared at the end of six or seven days. When it was injected after the production of polycythemia, the concentration of hemoglobin and the number of red cells decreased temporarily.

Baron and Baron [135] expressed the belief that the polycythemia described was due to inhibition of the respiratory function of immature red blood cells by cobalt, and that for this reason the cells appeared in the general circulation as mature nonrespirational cells, being replaced in the marrow by new cells. Thus, the addition of cobalt sulfate in vitro to suspensions of red cells from animals with polycythemia of this type was followed by marked inhibition in respiration, an effect not observed in the red cells of normal rabbits.

Cevitamic acid seems definitely to stimulate normal respiration of the red cells and acts therefore in maintaining the level of red cells in the circulating blood. This effect has been reported also in certain instances of anemia.

That cevitamic acid may accelerate the proliferation of monocytes is indicated by the experiments of Baker.[135] It may be that the influence of vitamin C on resistance in certain infections is in part explainable on this basis.

Baker [138] studied the effect of additions of cevitamic acid on the growth of monocytes in tissue culture. The medium contained blood serum, dextrose, Witte's peptone, cystine, hemin, insulin, thyroxin, vitamins A, B and D and salts. The cevitamic acid solution was preserved with glutathione, which inhibits the oxidation of vitamin C. The glutathione-cevitamic acid mixture was added in sufficient quantity to make a total concentration of cevitamic acid in the medium of from 0.05 to 2.5 mg. per hundred cubic centimeters. In concentrations of from 0.1 to 0.5 mg. of the vitamin per hundred cubic centimeters, the cells showed less granulation than normal cells, and proliferation was

138. Baker, L.: Compt. rend. Soc. de biol. **121**:427, 1936.

much more rapid. This effect, however, was not manifest until the end of the second week. If greater concentrations were used, the cells remained transparent and showed no increase in rate of growth. Glutathione alone had little or no effect. A solution of cevitamic acid alone, prepared just prior to use, had marked stimulative action. Baker [138] expressed the belief that cevitamic acid is one of the substances necessary for multiplication of monocytes in vitro.

The scorbutic state interferes with the reparative processes following injury to bone or soft tissues, according to Wolbach and Howe.[139] They fed 12 guinea-pigs for ten days a diet deficient in vitamin C. Simple incisions were made in tissues and bone and the processes of repair observed. In one group, orange juice was added to the diet. In the other, the deficient diet was maintained. The sequences of repair as studied at two, four, six and nine days showed that, though the epidermis grew normally, defective granulation tissue formed in the subcutaneous wound. The wounds remained ulcerated in many cases. There was no difference in the mononuclear inflammatory response, in the foreign body giant cell reaction or in the proliferation of muscle nuclei in the scorbutic animals as compared with the controls. Healing of the wound, however, proceeded by avascular organization. Fibroblasts grew in and produced no collagen or only traces of it, though fibroglia fibrils formed. In the scorbutic guinea-pigs, the injury to the bone was not followed by new bone formation as in the normally fed animals. The gap was filled with fibroblast-like cells without a trace of collagen and unaccompanied by capillaries. When orange juice was given, new bone was readily formed in a fibrin-like matrix in immediate contact with the cut bone. In scurvy, there is complete absence of repair of bone, without the formation of collagen or bone matrix.

The effect of avitaminosis on regenerative processes following experimental injuries is of considerable importance in this discussion, since the mechanism of resistance is intimately linked to the mechanism of repair of injury. Ishido [140] observed that in guinea-pigs and rats maintained on vitamin-free diets, wound healing is definitely delayed as compared with that in animals fed a normal, adequate diet or animals partially starved on a normal diet. The adherence of the wound is poor, and improper new formation of connective tissue occurs. This leads to collection of fluid in the floor of the wound with cavity formation and necrosis, and secondary infection is frequent. Saitta [141] likewise observed that guinea-pigs fed a scorbutic diet during a period of sixteen days prior to injury showed impairment of cicatrization.

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139. Wolbach, S. B., and Howe, P. R.: Am. J. Path. **9**:275, 1933.
140. Ishido, B.: Virchows Arch. f. path. Anat. **240**:241, 1922.
141. Saitta, S.: Scritti biol. **4**:301, 1924.

These findings are in accord with the more accurate observations of Wolbach and Howe,[139] previously cited, on the failure of proper repair in scorbutic animals, due to a defect in the formation of intercellular cement substance. Similar observations were reported by Lauber.[142]

However, a diet rich in vitamin C had little effect on the rate of healing of experimentally produced wounds of guinea-pigs, according to Lauber,[142] though he admitted that he did not standardize the quantity of vitamin C used. Saitta[141] suggested that an application of vitamin C to wounds stimulated healing.

These experiments on wound repair suggest the importance of vitamin C in the reparative process subsequent to injury and inflammation.

SUMMARY

The scorbutic state is associated with a drop in natural resistance to spontaneous infection and to induced bacterial infection. The production of natural or immune antibodies is unaffected, with the possible exception of that of opsonins. An interference with the capacity for immunization to tetanus and diphtheria toxin may occur in scurvy. Skin sensitivity to diphtheria toxin and tuberculin is diminished, but this is associated with greater susceptibility of the animal to the toxic effect of parenteral injections of these poisons. Skin reactivity to drugs such as arsphenamine occurs in guinea-pigs fed a diet partially deficient in vitamin C. When the avitaminosis is sufficient to cause clinical manifestations of scurvy, the skin is no longer reactive.

A short period of vitamin C deficiency prior to the administration of the sensitizing dose of protein antigen increases the anaphylactic response to a shocking dose subsequently injected and shortens the period of incubation of anaphylactic sensitization. Extreme scurvy or periods of starvation may, under certain conditions, impair the response to anaphylaxis.

Experimentally, it has been observed that the natural resistance of scorbutic guinea-pigs to tuberculosis and to infections with the colon group of organisms is lowered. Chronic staphylococcic infections tend to inhibit the deleterious effects of scurvy, apparently owing to the fact that such bacteria are capable of producing cevitamic acid in their growth.

By its profound influence on tooth growth and structure and gingival conditions, a deficiency of vitamin C may lead to increased susceptibility of the gums to infection and lower resistance of the tooth pulp to injury. In this way, the opportunity for the creation of dental foci of infection is greatly increased. Since such dental injury occurs very

142. Lauber, H. J.: Beitr. z. klin. Chir. **157**:244, 1933.

early in clinical scurvy and in so-called latent scurvy, the influence of vitamin C deficiency in infection is greater than is at first apparent.

Clinically, the scorbutic state and latent scurvy are associated with marked increase in the incidence of infections of the respiratory tract, of tuberculosis and of pyelitis in infants. The latent scorbutic state is often converted into frank scurvy by infections, and under such conditions hemorrhagic phenomena are frequent. This suggests that during the course of infections a greater demand is made on available vitamin C because of increase in the rate of tissue respiration.

The possible importance of vitamin C deficiency in susceptibility to rheumatic infections has been suggested, and experimental lesions said to be analogous to lesions observed in rheumatic endocarditis, myocarditis and arthritis have been produced by injecting cultures of several organisms into guinea-pigs suffering with latent scurvy.

An excess of cevitamic acid increases the resistance of guinea-pigs to diphtheria toxin, to tuberculin, to anaphylaxis and to trypanosoma infections and of monkeys to the virus of poliomyelitis. Its value experimentally and clinically in the treatment of tuberculosis in guinea-pigs and in man is not established, though it is possible that an excess of the vitamin may modify the incidence of intestinal complications. The prophylactic or therapeutic value of an excess of vitamin C needs further confirmation through experimentation. It seems logical that in the course of infectious diseases the increased rate of cellular oxidation would demand a greater amount of cevitamic acid than is needed normally.

The influence of vitamin C on resistance is dependent in part on its importance in the production of intercellular cement substance. However, the wide distribution of vitamin C in the body, its chemical properties and its influence on tissue respiration suggest that its rôle in natural resistance to infection is dependent on its physiologic importance in oxidation-reduction processes in cellular metabolism.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths etc.—Kenneth F. Maxcy, professor of preventive medicine and public health in the University of Minnesota, has been appointed professor of bacteriology in the Johns Hopkins University School of Hygiene and Public Health, to succeed William W. Ford, who has reached the age of retirement.

Leo Loeb, professor emeritus of pathology in the Washington University School of Medicine, George R. Minot, professor of medicine in the Harvard Medical School, and E. W. Goodpasture, professor of pathology in the Vanderbilt University School of Medicine, have been elected members of the National Academy of Sciences.

The Laveran Gold Medal of the Société de Pathologie Exotique of Paris has been awarded to A. W. Sellards, associate professor of tropical medicine in the Harvard Medical School, for his work on a vaccine against yellow fever.

Society News.—The annual meeting of the American Association of Public Health will be held in New York, October 5 to 8. The program of the laboratory section will deal with pertinent problems in public health and preventive medicine.

At its last annual meeting, the American Society for Experimental Pathology elected C. Phillip Miller president, Morton McCutcheon vice president, Paul R. Cannon secretary-treasurer and Shields Warren member of the council. The next meeting of the society will be held in Baltimore in conjunction with the Federation of American Societies for Experimental Biology.

American Leprosy Foundation.—The board of the Leonard Wood Memorial for the Eradication of Leprosy has authorized the general use of the name American Leprosy Foundation to indicate its activities.

Cancer Council.—Formation of a cancer council, to bring together the fields of education, therapy and research on cancer and to serve as a liaison agent between the outstanding authorities on cancer and the lay public through the medium of the press and radio, has been announced by Clarence C. Little, managing director of the American Society for the Control of Cancer. Members of the council include: Frank Adair, appointed by the regents of the American College of Surgeons; Karl Kornblum, appointed by the president of the American Roentgen Ray Society; Clarence C. Little and James B. Murphy, appointed by the council of the American Society for Cancer Research, and James Ewing and Burton T. Simpson, appointed by the directors of the American Society for the Control of Cancer. The first meeting of the council was held on March 5.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Acute Postoperative Necrosis of the Liver. J. E. Sutton Jr., Am. J. M. Sc. **192**:219, 1936.

The so-called high temperature liver death syndrome is a clinical entity characterized by rapid elevation of the temperature, falling blood pressure, circulatory collapse, coma and death with fever as high as 109 F. within from thirty-six to forty-eight hours after operation. The essential lesion in the liver is diffuse central necrosis of the lobules. A similar clinical course and similar hepatic changes have been produced in six of fourteen dogs by ligation of the hepatic arteries. In eight dogs which did not die there was adequate collateral circulation to all lobes of the liver and there was no hepatic necrosis. The similarity of the clinical course and the pathologic changes in the liver of the dog to those in man makes it reasonable to apply a pathologically descriptive phrase to this postoperative complication — "acute postoperative necrosis of the liver."

From the Author's Summary.

Regeneration of Autoplastic Splenic Transplants. D. Perla, Am. J. Path. **12**:665, 1936.

Transplanted splenic tissue undergoes rapid degeneration within the first twenty-four hours. A thin zone of viable reticular cells survives. There is little inflammatory reaction about the transplant. By the third day there is marked proliferation of the reticular cells at the outer zone, and streamers of cells are seen penetrating the necrotic center of the transplant. In some instances the entire transplant is replaced by reticular cells, which have formed the pattern of a sinus organ. By the third or fourth day lymphocytes appear irregularly in clusters, and apparently all stages of lymphoid cell formation are observed. By the sixth day the outer two thirds of the transplant are replaced by reticular elements arranged in an intricate mesh as in the adult spleen, with sinus formation, and ill defined clusters of lymphocytes are present. Complete regeneration of large transplants occurs from the twelfth to the twenty-first day, and the morphologic structure of adult splenic tissue is apparent, with a well developed capsule and trabeculae. These observations suggest that the reticular cell of the adult spleen retains its potentiality for differentiation and may be the precursor of the structural elements of the spleen.

From the Author's Summary.

Prolonged Application of Follicular Hormone on the Rabbit Uterus. B. Zondek, J. Exper. Med. **63**:789, 1936.

In rabbits follicular hormone under physiologic conditions produced hyperplasia of the uterine muscular wall and proliferation of the uterine mucous membrane. Prolonged application of large doses of the hormone brought about the following changes: (*a*) hyperemia of the myometrium and endometrium, with occasional scanty extravasal hemorrhages; (*b*) glandular-cystic hyperplasia of the endometrium; (*c*) infarct-like necrosis of the myometrium; (*d*) aseptic suppuration in the uterine cavity. These four processes can sometimes be found simultaneously in the same uterus, but they occur more frequently in sequence. While follicular hormone applied in physiologic doses has a stimulating effect, prolonged applica-

tion of large doses destroys the uterus. The effects described were to be observed only in the rabbit, not in the rat. This illustrates the fact that hormone reactions may vary in different species. FROM THE AUTHOR'S SUMMARY.

SOME EFFECTS OF OVARIECTOMY DURING THE PERIOD OF DECLINING REPRODUCTIVE POWERS IN MICE. W. S. MURRAY, J. Exper. Med. **63**:893, 1936.

Cessation of breeding and castration of female mice approaching the period of declining reproductive power have at least two effects: The average expectation of life is appreciably increased. The incidence of tumor is markedly decreased, especially in the older age groups. FROM THE AUTHOR'S SUMMARY.

THE EFFECT OF AVITAMINOSIS-A ON THE PROSTATE. R. A. MOORE and J. MARK, J. Exper. Med. **64**:1, 1936.

In the white rat deficiency of vitamin A alone is associated with atrophy of the testis and accessory sexual glands. This appears to indicate some disturbance in the hypophysial-gonadal-prostatic hormonal relationships. All of the vitamins known to be necessary to the rat except vitamin A were present in the diet of the animals studied. Deficiency of vitamin A in these rats was associated with foci of inflammation and epithelial metaplasia in the prostatic acini and vesicular ducts entirely similar to that reported in other organs. Focal metaplasia and inflammation of the prostate is occasionally encountered in patients with extreme inanition associated with stenosis of the esophagus. It seems probable that this lesion is due to deficiency of vitamin A. FROM THE AUTHORS' SUMMARY.

THE CULTIVATION OF TISSUES FOR PROLONGED PERIODS IN SINGLE FLASKS. R. C. PARKER, J. Exper. Med. **64**:121, 1936.

Fragments of breast muscle from a 12 day old chick embryo have been kept alive in single flasks for an entire year without being transferred. The nutrient materials were supplied by frequent applications of adult fowl serum diluted with Tyrode solution. When fragments of fixed tissues are cultivated in serum, cell multiplication and cell death are both reduced to an extremely low level.

FROM THE AUTHOR'S SUMMARY.

STUDIES OF GALLBLADDER FUNCTION. C. RIEGEL, I. S. RAVDIN, C. G. JOHNSTON and P. J. MORRISON, Surg., Gynec. & Obst. **62**:933, 1936.

In a previous study the authors have shown that when the human gallbladder becomes damaged there occurs a change in the composition of the gallbladder bile. The present paper is concerned with the composition of the bile and calculi removed from patients exhibiting various stages of gallbladder disease. It is now generally agreed that the normal gallbladder changes the composition of the hepatic bile in a consistent manner by absorbing water and with it chloride and bicarbonate so that the concentrations of these substances normally are only fractions of their concentrations in the hepatic bile. At present there is strongly suggestive evidence that normally small amounts of calcium and of bile salt are absorbed during the concentration of bile and that bile pigment and cholesterol remain quantitatively in the gallbladder during this concentration. The data presented here demonstrate that in the presence of cholecystitis the absorbing function of the gallbladder becomes altered. The chloride concentration of the bile in the sac rises owing to failure of the gallbladder to remove the chloride brought to it in the hepatic bile or to chloride coming into the bile with any fluid passing into the gallbladder from its wall or to a combination of these factors. The decrease in the calcium concentration may also be due to the failure of the gallbladder to concentrate the hepatic bile or to the removal of calcium from the solution as calcium carbonate

by precipitation owing to the high carbon dioxide in the gallbladder bile. The most constant indication of a disturbance of function is a decrease in the bile salt concentration, for with increasing evidence of a disturbance of gallbladder function there is nearly uniformly a parallel decrease in the values for bile salt. It was not possible to find any parallelism between the conventional pathologic diagnosis and the changes in the chemical composition of gallbladder bile. No consistent differences were found between the composition of bile from gallbladders containing cholesterol stones and bile from sacs in which there were stones of the mixed type. There was also no constant direct relationship between the pathologic classification of the gallbladder and the chemical composition of the bile, although certain gross differences between the biles of the various pathologic groups were demonstrable. The cholesterol concentration varied considerably but was consistently higher in pregnant women and lower in the "calculus nonvisualized" group with a low bile salt content. In the pregnancy group the bile salt concentration was apparently sufficient to keep a high concentration of cholesterol in solution in the bile. These data indicate certain trends in the chemical composition, demonstrating that in the various stages of cholecystitis, as they are now conventionally given, the degree of impairment of the normal mechanism of concentration varies widely. The mere presence of calculi does not always indicate that this mechanism is more pathologic than it may be in a gallbladder which does not contain stones but is the site of inflammation.

WARREN C. HUNTER.

HISTOLOGIC CHANGES IN THE SEX ORGANS OF SPAYED RATS INDUCED BY TESTOSTERONE AND ESTRONE. V. KORENCHEVSKY and M. DENNISON, J. Path. & Bact. **43**:345, 1936.

In the sex organs of sixty spayed female rats the effects of administration of an androgenic substance (testosterone) and an estrogenic factor (theelin) were studied and the changes compared with those seen at different periods of the normal sexual cycle. The results are compared with, and corroborate, those obtained in the authors' previous experiments in which twenty-six normal and fifty spayed rats were given injections of the estrogenic substance. Various physiologic and pathologic effects are described, and in several cases these were present simultaneously. The chief pathologic effects were either an incomplete return to the normal condition or a vaginal change typical of or similar to one sexual state of the cycle, accompanied by a uterine change belonging to another and an abnormal hypertrophy of the preputial glands. The condition of the vagina and uterus obtained with the doses of the estrogenic substance was found to differ in some respects from the condition of the vagina and uterus of normal rats during estrus. The androgenic substance injected alone into spayed rats brought about an incomplete return to the normal size and histologic structure of the uterus and vagina and, with high doses, a special type of mucification of the vaginal epithelium. This substance and the estrogenic substance, when injected simultaneously into spayed rats, showed cooperative activity and produced, with high doses, changes in the vagina similar to those seen during pregnancy and changes in the uterus somewhat similar to those seen during pro-estrus. The changes produced by testosterone extract in the sex organs of females were similar to those obtained after the injection of androsterone-diol and differed in some respects from those produced by androsterone.

FROM THE AUTHORS' SUMMARY.

CERTAIN EFFECTS OF PULMONARY GAS EMBOLISM. I. SINGH, J. Physiol. **87**:11, 1936.

Pulmonary gas embolism was accomplished by injecting oxygen intravenously. The effects consisted in: an acceleration of circulation through the lungs as a result of increased cardiac output and blockage of the pulmonary arterioles; an

initial diminution of oxygen consumption; a 5 per cent increase of metabolism. Relief from spasm due to embolic asphyxia and from disordered respiration was obtained by administering epinephrine.

PATHOGENESIS OF EXPERIMENTAL ULCER. L. DELOYERS, Arch. internat. de méd. expér. **11**:2, 1936.

Anastomosis of the accessory pancreatic duct into the ileum in dogs did not lead to the formation of an ulcer. Choleocysto-ileostomy with ligature of the common bile duct provoked the formation of an ulcer in the duodenum very frequently and in the stomach rarely. The gastric chemistry, qualitative and quantitative, showed little difference before and after the operation of Mann. Animals subjected to extensive gastrectomy showed no intestinal ulcers. Acidity played an important rôle and mechanical factors were secondary in the pathogenesis of intestinal ulcers. Cachexia and malnourishment played a vital rôle. The integrity of the gastric and intestinal mucosa is dependent on normal function of the liver and gallbladder. Biliary obstruction, Eck fistula, ligature of the common bile duct and hepatitis provoke modifications in gastric chemistry and often lead to typical ulceration. Suppurative hepatitis and injection of cinchophen cause ulcers. Glycuronic acid is thought to be an antiulcerous principle, and abundance of mucin in the gastro-intestinal tract is thought to prevent the development of ulcers. Camphor administered in daily doses caused atrophy of the mucosa and the appearance of perforating ulcers in dogs.

FREDERICK STENN.

THE COURSE OF VITAL STORAGE IN LYMPH NODES. L. H. KETTLER, Virchows Arch. f. path. Anat. **297**:40, 1936.

Nordmann had claimed that the course of vital storage in lymph nodes, i. e., whether the material is stored first in the sinus endothelium or in the reticulo-endothelium of the interfollicular tissue and finally of the follicles, depends on the quantity of material carried to the nodes by the lymph stream. Wätjens and Eilers, on the other hand, had claimed that the course of storage depends on the time elapsed since the introduction of the material, which was india ink. Kettler used homologous blood, iron, trypan blue, olive oil, croton oil and cod liver oil. The rabbit was the experimental animal. He found that the various substances used, except olive oil, are stored first in the sinus endothelium, and are then transferred to the reticulo-endothelium of the lymphoid tissue. Olive oil filled the sinuses, then passed to the lymphoid tissue, and disappeared without evoking any reaction in the nodes.

O. T. SCHULTZ.

ORIGIN OF FAT AND LIPOIDS IN TISSUE CULTURES. E. RIX, Virchows Arch. f. path. Anat. **297**:93, 1936.

Intracellular lipoid substances make their appearance in tissue cultures within from twenty-four to forty-eight hours after explantation. During a first phase of from one to four days visible fat is not proportional to the quantity of fat detectable chemically, which has led some observers to assume that the fat is formed from protoplasmic carbohydrates or proteins. During a second phase, of the fourth to ninth days, characterized by ageing and death of cells, visible fat increases, presumably as the result of necrobiosis and autolysis. Rix determined quantitatively by chemical methods the total fat and cholesterol at the time of explantation and on the fourth day thereafter. Lipoid phosphorus was determined at the beginning and on the fourth and eighth days of cultivation. His failure to detect any increase in these substances leads him to conclude that the intracellular fat of tissue cultures is the result of fatty infiltration and not of fatty transformation, and that a synthesis of cholesterol does not occur.

O. T. SCHULTZ.

Pathologic Anatomy

ENCAPSULATION OF FOREIGN MATERIAL IN THE PERITONEAL CAVITY. A. J. WALLINGFORD, Albany M. Ann. **55**:78, 1936.

Poppy seeds are not absorbed by the peritoneum but are encapsulated. The seeds gravitate generally to the dependent portions of the peritoneal cavity. Many are enmeshed in the omentum. The process of encapsulation proceeds through definite stages: the fibrin stage, the organizing stage and the fibrous stage. The process of disposal of this type of foreign body is similar to that described by Sampson for one group of the foreign body type of peritoneal cancer implant, the only difference being the inert nature of the poppy seed.

FROM THE AUTHOR'S SUMMARY.

THE PARADOX OF CHIARI'S NETWORK. W. M. YATER, Am. Heart J. **11**:542, 1936.

Venous valves of the right atrium, the eustachian and the thesbesian, may be considered normal parts of the adult heart. They are remnants of the right venous valve of the sinus venosus of the embryo and serve no useful purpose in extra-uterine life. Chiari's network, a reticulum of fibrous threads in connection with these valves, with attachments to the crista terminalis and Lower's tubercle, occurs probably in from 2 to 3 per cent of otherwise developmentally normal hearts. Chiari's network produces no symptoms or signs. This network may be the origin of thrombi which may give rise to pulmonary emboli, or it may ensnare emboli from some systemic vein and prevent sudden fatal pulmonary embolism (the paradox of Chiari's network). A case is reported in which death eventually occurred from embolic pulmonary infarcts and low grade infection and in which a network of Chiari had ensnared an embolus 11.5 cm. long, originating from an asymptomatic thrombus which had formed in a large vein. The patient apparently had thrombophilia, since he had just previously had coronary arterial thrombosis and also, later, mural thrombi in the right atrium and ventricle. The case illustrates the rapidity with which hypertrophy of the right atrium and ventricle may occur.

FROM THE AUTHOR'S SUMMARY.

THYMOMA AND THYMIC HYPERPLASIA IN MYASTHENIA GRAVIS. E. H. NORRIS, Am. J. Cancer **27**:421, 1936.

Four cases of myasthenia gravis with autopsies are reported. In two of these a high degree of hyperplasia of the thymus was observed; the other two showed no gross evidence of thymic involvement. Fifty per cent of the reported cases of myasthenia gravis in which autopsies were made have shown some form of thymic involvement. Pathologic conditions of the thymus which have been regarded as benign tumors have been found in half of the cases of myasthenia gravis in which demonstrable changes in the thymus were recognized. Such conditions Norris has interpreted as conditions of extreme epithelial hyperplasia. Pathologic conditions which have been regarded as enlargement or persistence of the thymus have been found in half of the cases of myasthenia gravis in which demonstrable changes in the thymus were recognized. These he has interpreted as conditions of moderate epithelial hyperplasia.

FROM THE AUTHOR'S SUMMARY.

ALTERATIONS IN THE LIPOID CONTENT OF THE ADRENALS IN INFANCY AND IN CHILDHOOD. M. L. MENTEN and M. P. SMITH, Am. J. Dis. Child. **52**:54, 1936.

Wide variations in the lipoid content of the cortex were observed in the adrenals from seventy patients. The most marked depletion occurred in infections of long duration. Comparison of the vitamin C concentration of one adrenal with the histochemical lipoid content of the second adrenal in nine cases showed a corresponding reduction of the two substances.

FROM THE AUTHORS' SUMMARY.

DERMATOMYOSITIS. A. WOLF and S. L. WILENS, Am. J. Path. **12**:235, 1936.

Dermatomyositis is an inflammatory, often degenerative disease of the skin and striated musculature. The skin reaction resembles erythema, erythema nodosum, erythema multiforme, erysipeloid, roseola or urticaria; a nonpitting edema develops, then scaling, and the involved tissues become brownish, leathery, tight, atrophic and fixed to the underlying tissues, the process ending often in scleroderma. The papillae are flattened, the cells of the basal layer vacuolated, the dermis edematous and its collagen fibers swollen and semihyaline. Perivascular and periglandular infiltration by round cells occurs, chiefly lymphocytes and monocytes collecting, especially beneath the epidermis. Finally, desquamation of the cornified epidermis occurs with disappearance of the papillae, increase of the collagen in the corium and fibrosis of the fatty tissue. The muscles, most conspicuously the skeletal musculature and the muscles of deglutition and respiration, become dull, gray-yellow or pale red, firm and rubbery or soft and doughy, and hemorrhagic and friable. Fibrosis occurs rarely, with calcification. The endomysium and perimysium show edema with diffuse infiltration by round cells, including lymphocytes, large mononuclears, plasma cells, polymorphonuclear leukocytes and occasional mast cells. The muscle fibers undergo loss of striation, fragmentation of fibrils, and granular, hyaline or waxy degeneration with vacuolation or rupture. Bleb formation in the sarcolemma occurs frequently. The possibility that a virus is the etiologic agent is considered. Two cases are reported. In one the muscles showed creatine diminished.

FREDERICK STENN.

MITRAL STENOSIS WITH INTERAURICULAR INSUFFICIENCY. S. SAILER, Am. J. Path. **12**:259, 1936.

Mitral stenosis with interauricular septal insufficiency has been reported in twenty-seven cases. The characteristic features are marked hypertrophy and dilatation of the right side of the heart, thickening, sclerosis and stenosis of the mitral orifice and a defect of variable size in the auricular septum. In the case described the right ventricle formed the greater portion of the anterior surface of the heart, the apex reaching the sixth left rib in the anterior axillary line. The right ventricular wall measured from 15 to 17 mm. in thickness at the base and the left ventricular wall 20 mm. The mitral orifice measured 9 cm. in diameter; the leaflets were sclerotic and the chordae tendineae fibrotic and shortened. Below the mouth of the auricular orifice, just above the septal flap of the tricuspid valve, was a septal defect measuring 3 cm. in diameter. The defect is explained on the basis of a developmental arrest of the primary or secondary foramen ovale or a reopening of a secondary foramen by acquired hypertrophy and dilatation of the auricles. Rarely does such a defect result from endocarditis.

FREDERICK STENN.

ARHINENCEPHALY WITH EXTREME EVERSION OF THE END-BRAIN. H. KUHLENBECK and J. H. GLOBUS, Arch. Neurol. & Psychiat. **36**:58, 1936.

Kuhlenbeck and Globus describe the brain of an infant who died on the fourth day after normal delivery. The only symptom was marked cyanosis. The end-brain showed incomplete division of the hemispheres and a horseshoe-shaped mass with a dorsal defect which opened into a large, unpaired hydrocephalic cavity. Macroscopic studies of the stained serial sections revealed absence of the occipital lobes, incomplete development of the temporal lobes, rudimentary development of the corpus striatum and corpus callosum and absence of the pyramids, olfactory bulbs, olfactory tracts, trigonum olfactorium, some olfactory striae and of the diagonal band of Broca. The optic thalamus, optic tracts, optic chiasm and geniculate bodies were fairly well developed; the cranial nerves and the brain stem were normal. Microscopically there was absence of corticopontile and corticospinal fibers, gigantopyramidal and postcentral areas and internal granular layer of the cortex. The cause of the malformation was very likely arrest or retardation of the development of the end-brain as in cyclopia, in which, however, there are also defects in the eyes and optic tract.

GEORGE B. HASSIN.

CHRONIC OCCLUSION OF THE PORTAL VEIN. J. P. SIMONDS, Arch. Surg. **33**:397, 1936.

A case of chronic occlusion or stenosis of the portal vein is reported, in which the favorable results which might have been expected from splenectomy were defeated by the presence of an unsuspected collateral circulation of an exceedingly rare type, namely, a large varicose communication between the splenic and the left renal vein. Chronic thrombosis of the portal vein occurs in one of two forms: Either a mural or incompletely obturating thrombus is organized with relatively little or no recanalization or the vein and adjacent tissue are transformed into a cavernous or angiomatous mass. There is a sharp difference of opinion as to whether the cavernous or angiomatous type is the result of thrombosis or a malformation or a true neoplasm. Those two forms differ, relatively, in the symptomatology and in the mode of death. The American and English medical literature contains few references to the subject of chronic occlusion of the portal vein, although this lesion occurs with sufficient frequency to justify its consideration in the differential diagnosis in all obscure cases of gastric hemorrhage or ascites.

FROM THE AUTHOR'S SUMMARY.

THE BILIRUBIN CONTENT OF BLOOD AND THE UROBILINOGEN CONTENT OF URINE IN DIABETES MELLITUS. I. M. RABINOWITCH, Brit. J. Exper. Path. **17**:249, 1936.

Attention is drawn to the high incidence of hyperbilirubinemia and of excess of urobilinogen in the urine in diabetes. Assuming that, in the absence of biliary obstruction or hemolysis, an excess of bilirubin in the blood is due to hepatic disease and that an excess of urobilinogen in the urine is due to hepatic disease in the great majority of cases, the data cited afford further proof of the high incidence of disease of the liver in diabetes.

FROM THE AUTHOR'S SUMMARY.

THE PARS INTERMEDIA OF THE HYPOPHYSIS IN THE ANTHROPOID APE AND MAN. A. PLAUT, J. Anat. (pt. 2) **70**:242, 1936.

In pursuing previous studies of the rudimentary character of the pars intermedia of the hypophysis of the anthropoid ape and man, a number of hypophyses from monkeys and apes were examined (cebus, woolly monkey, orang-utan, chimpanzee, gibbon). The volume of the pars intermedia in relation to the other portions of the hypophysis was determined by the paper cutting and weighing method. This method is very exact. In the ape the hypophysis was found as rudimentary as in man and in the previously examined chimpanzee and orang-utan. The functions of the intermediate lobe in man and ape probably have been taken over by the anterior lobe. The possible relations between the morphology of the hypophysis, the external form of the body and its phylogenetic development are discussed. The aquatic mammals, especially the whales, differ in the structure of the hypophysis from all other mammals, as shown by Wislocki. Further work on the hypophysis of the whale is in progress.

FROM THE AUTHOR'S ABSTRACT.

CORPORA AMYLACEA IN NERVE CELLS. L. MARCHAND, Ann. d'anat. path. **12**:1, 1935.

In the brains of two patients with epilepsy, Marchand found within ganglion cells round bodies giving the characteristic staining reactions of corpora amylacea. Such intracellular corpora amylacea are rare, having previously been reported in only three cases. There are two forms, homogeneous and concentric, or lamellar, the former being the early stage. The bodies probably represent a very slow degenerative change in the ganglion cell.

PERRY J. MELNICK.

PATHOLOGIC-ANATOMIC STUDY OF ENDOCARDITIS. G. ALBOT and A. MIGET, Ann. d'anat. path. **12**:13, 1935.

From an analysis of the gross and microscopic lesions of endocarditis, Albot and Miget conclude that there is no clearcut dividing line between rheumatic (benign) and malignant endocarditis; that a series of intermediary forms can be recognized between verrucous and sclerotic lesions, on the one hand, and vegetative and ulcerative forms on the other. The character of the initial endocarditic process may serve to differentiate the two forms, but the clinical characteristics of the two diseases are a better guide.

PERRY J. MELNICK.

OSTEOGENESIS IN "MARBLE BONE" DISEASE. O. H. DIJKSTRA, Ann. d'anat. path. **12**:131, 1935.

Dijkstra studied a case of "marble bone" disease (osteopetrositis; Albers-Schönberg disease) in a 3½ year old boy who had severe anemia, for which splenectomy had previously been performed. The spleen had the histologic appearance of bone marrow, even including megakaryocytes. The bones (rib, vertebrae, skull) showed histologic evidence of delayed absorption in the center while peripheral growth continued. This disturbance of bone development is considered by the author to be the underlying cause of the disease.

PERRY J. MELNICK.

INTESTINAL OBSTRUCTION IN AN INFANT DUE TO AN UNUSUAL CAUSE. A. PERROT and L. DANON, Ann. d'anat. path. **12**:157, 1935.

In a 15 day old infant with symptoms of intestinal obstruction and fecal vomiting, operation disclosed no mechanical obstruction. At autopsy there was found marked hypertrophy of the small intestine, especially of the ileum (resembling Hirschsprung's disease of the colon). The hypertrophy was due entirely to a thickening of the muscular layer. The terminal portion of the ileum and the proximal portion of the colon were not hypertrophic but were found to show almost complete absence of the plexus of Auerbach. The authors believe that either paresis or spasm of this segment resulted in the intestinal obstruction, with secondary hypertrophy of the bowel above. They believe that this segment of the intestinal tract (terminal portion of the ileum and proximal portion of the colon) may have separate innervation, so far not differentiated by the physiologists.

PERRY J. MELNICK.

CONCERNING SENILE MACULAR PIGMENTATION. G. MIESCHER, L. HÄBERLIN and L. GUGGENHEIM, Arch. f. Dermat. u. Syph. **174**:8, 1936.

The authors examined twenty-nine specimens of acquired pigmented macules in old persons. Clinically the lesions were divided into small macules (senile freckles), large macular lesions and leukomelanoderma. The lesions became more frequent with age; of persons between 80 and 100 years of age, 97 per cent showed pigmented macular lesions. The histologic picture usually was that of varying amounts of atrophy with irregular distribution of pigment in ordinary basal cells. In two cases multiplication of dendritic melanoblasts in the basal layer heralded the onset of precancerous melanotic lesions. A large number of pigmented macules, especially on the hands, appeared on section to be rudimentary pigmented senile warts. Differentiation of ordinary harmless pigmented lesions from precancerous melanotic lesions and rudimentary senile warts can be made only microscopically.

S. W. BECKER.

PERFORATION OF THE INTRAVENTRICULAR SEPTUM. S. T. MAHRBURG, Frankfurt. Ztschr. f. Path. **47**:554, 1935.

A 55 year old man who had experienced a single sudden attack of pain in the chest and dyspnea died as a result of heart failure one month after this attack.

Autopsy revealed marked arteriosclerosis with coronary sclerosis. The descending branch of the left coronary artery was occluded by a thrombus, and the descending branch of the right coronary artery was occluded by arteriosclerotic plaques. There was a large scar in the interventricular septum involving almost its entire width and extending over an area 4 by 5 cm. in the longest dimensions. The scar revealed four perforations, which were separated from one another by connective tissue. The perforations had led to a communication between the right and left ventricles. The belief is expressed that the infarction of the septum occurred about one month before the death of the patient and that the resulting perforation occurred gradually.

OTTO SAPHIR.

CHANGES IN THE SPINAL CORD AND POSTERIOR ROOT GANGLIONS IN SEPSIS. S. S. WAIL, Virchows Arch. f. path. Anat. **295**:414, 1935.

The spinal cord and posterior root ganglia were examined histologically in thirty fatal cases of sepsis. Swelling of the walls of small blood vessels, sometimes with thrombus formation, fibrinoid necrosis, perivascular cellular proliferation and hemorrhage were observed. Wail considers the changes noted hyperergic.

O. T. SCHULTZ.

PROGRESSIVE LIPOMATOSIS OF THE MUSCULATURE WITH LIPOCALCINOGRANULOMATOSIS. O. TEUTSCHLAENDER, Virchows Arch. f. path. Anat. **295**:424, 1935.

Teutschlaender presents a report of a unique condition that has been under observation for five years. At the age of 3 years a painless swelling was noted below the right scapula. Its rather rapid growth led to a diagnosis of sarcoma and to removal. A similar swelling developed in the same situation on the left side, and since that time there has been a progressive formation of symmetrical swellings of the scapulohumeral group of muscles. Larger swellings reach the size of a fist; they become partly fluctuant and break down with the formation of discharging sinuses. Gross and microscopic examination of removed tumors revealed a chronically inflammatory granulation tissue with cavernous spaces. The latter were filled with partly calcified granular fatty detritus and were surrounded by granulation tissue containing foreign body giant cells. Teutschlaender believes that the primary condition was a progressive symmetrical lipomatosis of the musculature. This heredodegenerative process, he thinks, may be a scapulohumeral form of the lipodystrophia progressiva of Simons. Spontaneous necrosis of the fat that replaces the muscle is followed by deposition of calcium and formation of foreign body granulation tissue. Endarteritis obliterans was observed in the inflammatory tissue. Teutschlaender expresses the opinion that such vascular inflammatory change on an infectious basis resulting from otitis media early in life may be the initiating factor in the fat necrosis.

O. T. SCHULTZ.

ACUTE DEGENERATIVE MYOCARDITIS IN INFLUENZA. F. ROULET, Virchows Arch. f. path. Anat. **295**:438, 1935.

A barracks epidemic of influenza was followed by the sudden death of two patients aged 20 and 25 years two and three weeks after the onset of the acute attack. Microscopic examination revealed localized and diffuse changes in the myocardium, characterized by swelling of the contractile substance, vacuolation of muscle fibers and granular disintegration of the latter. The degenerative process was followed by slight proliferation of the finer supporting tissue with moderate leukocytic infiltration. Roulet notes that clinically heart involvement has been more frequent and more severe in the recent minor epidemics than in the pandemic of 1918. He surmises that secondary or mixed infection may be a more important factor in this process than the primary incitant of influenza.

O. T. SCHULTZ.

MORGAGNI'S SYNDROME—INTERNAL FRONTAL HYPEROSTOSIS, VIRILISM AND OBESITY. F. HENSCHEN, Hygiea **98**:65, 1936.

In 1761 Morgagni described frontal hyperostosis associated with virilism and obesity in a woman 75 years old. Henschen points out that such hyperostosis is not unusual in women after the age of 45. It was present in about 40 per cent of 170 women aged 47 years or more whose skulls he studied carefully. The condition occurs only very rarely in men. In women the development of frontal hyperostosis seems to be related to the climacterium, when the physical constitution may shift toward the masculine. The hyperostosis does not give rise to any clinical symptoms so far as known; it may be of interest in paleopathology and may be significant in the determination of age and sex in skulls and skull fragments.

Microbiology and Parasitology

TUBERCULOUS BACILLEMIA. H. J. CORPER and C. B. VIDAL, Am. Rev. Tuberc. **32**:575, 1935.

A fine suspension of virulent tubercle bacilli of the human, bovine or avian strain injected intravenously into guinea-pigs, rabbits, chickens or dogs disappears from the circulation rapidly when injected in amounts of 0.001 or 0.000,001 mg., even though these amounts are capable of producing generalized progressive organic tuberculosis. When large amounts (1 mg.) of a fine suspension of tubercle bacilli highly virulent for the animal in question are injected, the bacilli may remain in the circulation until the early acute death of the animal from the overwhelming dose, but there is no evidence to support the claim that they multiply in the circulation. If analogous amounts (1 mg.) of a fine suspension of bacilli avirulent for the animal in question are injected, the bacilli disappear from the circulation, but they may be found present for a week and possibly irregularly after that time, to disappear ultimately. There is a certain parallelism between the reactions of the various animal species to the intravenous injection of virulent tubercle bacilli in regard to the persistence of the bacilli in the blood after large doses, but there is a striking difference in organic susceptibility between these various animals to intravenous infection. From these observations and numerous previous observations in man, it appears that tuberculosis is not commonly bacillemia in the sense that the tubercle bacilli frequently find residence or multiply in the circulation. The bacilli can be found in the blood rarely in terminal conditions, and embolic dissemination can occur occasionally and intermittently in the course of the disease, which accounts for the multiple organic metastatic foci—an occasional embolemia.

FROM THE AUTHORS' SUMMARY.

THE SIGNIFICANCE OF FUSIFORM BACILLI AND SPIRILLA IN GINGIVAL TISSUE. R. TUNNICLIFF, E. B. FINK and C. HAMMOND, J. Am. Dent. A. **23**:1959, 1936.

Smear preparations from ulceromembranous and pyorrheal gingivitis both show large numbers of fusiform bacilli and spirilla. Sections of pyorrheal tissue stained with silver indicate that while a few fusiform bacilli may occasionally be found in the tissues, although not in such large numbers as in ulceromembranous conditions, the spirilla may invade living tissue in larger numbers, particularly in severe pyorrheal infections.

The fact that immunity tests (phagocytic) on patients with pyorrhea and ulceromembranous gingivitis both show definite reactions to cultures of fusiform bacilli suggests that this micro-organism may be of pathogenic significance.

FROM THE AUTHORS' SUMMARY.

HUMAN INFLUENZA VIRUS IN ARTIFICIAL MEDIUM. T. P. MAGILL and T. FRANCIS JR., J. Exper. Med. **63**:803, 1936.

The in vitro cultivation of strains of the virus of human influenza has been successfully conducted through a prolonged series of transfers. The cultivated

virus has retained the antigenic and immunologic properties which characterized the animal passage virus from which it was derived. The culture virus is still virulent for mice and ferrets; it is capable of inducing a state of active immunity in animals vaccinated subcutaneously or intraperitoneally; it elicits specific neutralizing antibodies in the serum of infected or vaccinated animals. The virus has been successfully cultivated to date only in the presence of oxygen; when conditions of reduced oxygenation are imposed by the use of a petrolatum seal with or without the addition of cysteine, multiplication of the virus is not supported. On the other hand, it has been possible to cultivate the virus in the medium of Li and Rivers in ordinary test tubes. This affords a greatly simplified procedure, since the interval between transfers may be prolonged. The results of neutralization tests with various serums are presented and discussed.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL ENCEPHALITIS (ST. LOUIS TYPE) IN MICE WITH HIGH INBORN RESISTANCE. L. T. WEBSTER and A. D. CLOW, J. Exper. Med. **63**:827, 1936.

The virus of encephalitis B (St. Louis) injected intracerebrally or intraperitoneally in maximum doses in resistant mice is distributed and is effective in a manner generally similar to that in susceptible mice. The minimal infecting dose is at least a thousand times greater in resistant than in susceptible mice, and virus injected into the brain tends to remain at a relatively low titer, persists for a few days and then disappears. Virus dropped in the nares is demonstrable and progresses in the brains of resistant mice as in those of susceptible mice but does not increase in titer beyond the fifth day, does not bring about fatal encephalitis and persists for at least four weeks. Lesions in the brains of resistant mice following nasal instillation of virus do not appear until the eighth day, reach a maximum at forty days and are still present, though resolving, at three months. The changes resemble those seen in the human disease and in other unnamed forms of encephalitis. The quantity of virus drops a thousand-fold when the virus is recovered from resistant mice and becomes noninfective by the nasal route. Passage through susceptible mice promptly restores its full titer.

FROM THE AUTHORS' SUMMARY.

EFFECT OF PURIFIED ENZYMES ON VIRUSES AND GRAM-NEGATIVE BACTERIA. M. H. MERRILL, J. Exper. Med. **64**:19, 1936.

Evidence is presented that some viruses behave like proteins in that they are inactivated by proteolytic enzymes, whereas others prove more or less resistant. Ten strains of living gram-negative bacteria resisted the action of purified trypsin and chymotrypsin, while the killed organisms were rapidly digested. Gram-positive bacteria, on the other hand, were resistant whether living or dead.

FROM THE AUTHOR'S SUMMARY.

SURVIVAL OF THE VIRUS OF POLIOMYELITIS IN THE ORAL AND NASAL SECRETIONS OF CONVALESCENTS. S. D. KRAMER and others, J. Exper. Med. **64**:173, 1936.

The positive detection of the virus of poliomyelitis in the nasal secretions of two children, sixteen and thirteen days after the onset of the disease, is described. Seven animals which had been inoculated with other concentrates became ill with symptoms and elevation of temperature suggestive of poliomyelitis from one to three weeks after inoculation, but without definite paralysis. In two of these animals which were put to death, the histologic observations were compatible with the diagnosis of poliomyelitis but were not typical. Of the serums of the five remaining animals, four failed to neutralize stock virus, whereas the fifth neutralized the virus on two different occasions. This serum was obtained from a monkey that had been inoculated with concentrated nasal secretion from a child forty-three days after the onset of illness. It is suggested that the present quaran-

tine period of three weeks is compatible with the available data. It is further suggested that the methods of procedure described may be useful in similar investigations.

FROM THE AUTHORS' SUMMARY.

THE EPIDEMIOLOGY OF LYMPHOCYTIC CHORIOMENINGITIS IN WHITE MICE. E. TRAUB, J. Exper. Med. **64**:183, 1936.

In a mouse colony in which lymphocytic choriomeningitis is endemic infection takes place either in utero or shortly after birth. Virus is discharged from infected mice with the nasal secretions and urine. In some mice the infection lasts for several months, and such carriers can transmit the disease to healthy mice by contact. The portal of entry appears to be the nasal mucosa rather than the gastro-intestinal tract. Mice infected in utero often die. The disease has persisted in the colony for at least fifteen months without change in its character. Mouse stocks differ in their susceptibility to contact infection, and the findings given in the paper could be reproduced only with a very susceptible stock. Wild mice (Mus musculus) can be infected by contact, although less easily than the white mice. The source of the infection in the colony has not been determined. The fact that the serum of the caretaker neutralizes the virus indicates that he has been infected. It seems likely that the virus went from him to the mice rather than vice versa. Other possible sources of infection are considered.

FROM THE AUTHOR'S SUMMARY.

INVESTIGATIONS OF VACCINE VIRUS. R. F. PARKER and T. M. RIVERS, J. Exper. Med. **64**:439, 1936.

Methods have been described by which the number of elementary bodies in a suspension can be estimated. It has been shown that by means of replicate counts, in which the Petroff-Hausser chamber was used, a high degree of accuracy can be attained. By means of the Gates densitometer, the number of elementary bodies in a suspension can be determined with a coefficient of variation of about 3 per cent. A method has been described by which the accuracy of the estimation of the infectious titer of a suspension can be increased without greatly enlarging the number of animals employed. This consists in selecting as the end-point that dilution of virus which theoretically on intradermal inoculation in a rabbit would lead to an equal number of positive and negative results. The statistical advantages of this method have been attested by the experiences of other laboratories. By the application of the methods described, there was shown to be a direct correlation between the number of elementary bodies and the number of infectious units of virus in a given suspension. At the mean of the distribution this ratio is as the logarithms 9.62 to 8. To extrapolate this curve in order to determine the number of elementary bodies in a single infectious unit, while tempting, is probably not justifiable. It must likewise be remembered that the data given apply to a particular strain of vaccine virus and that the number of infectious units has been determined by intradermal inoculation in rabbits. It appears also that this method may be of value in studies of the virulence of different strains of vaccine virus, since by its application one may determine not only the infectious titer of a suspension but its content of elementary bodies. In the agglutination reaction it was found that the optimum titer of serum was obtained when the test antigen contained from 2×10^9 to 0.05×10^{10} elementary bodies per cubic centimeter. Approximately 1.95×10^8 particles per cubic centimeter of suspension were required for the production of visible agglutination.

FROM THE AUTHORS' SUMMARY.

BEHAVIOR OF TYPHUS RICKETTSIAE IN TISSUE CULTURES. M. ASCHNER and I. J. KLIGLER, Brit. J. Exper. Path. **17**:173, 1936.

A technic is described for the cultivation of Rickettsia by using infected tissues for primary cultures. This technic has enabled Aschner and Kligler to study such problems as the manner of growth of these organisms and the time of their

appearance and of their disappearance in the infected animal. It was found that rickettsias grow in the serum of rabbits as well as in that of guinea-pigs and that a minimal concentration of serum of 1 : 12 is essential. Tyrode or Locke solution can be used—the bicarbonate acting as buffer. The favorable reaction is between the neutral point and p_H 7.6. Any endothelial membrane will serve as culture tissue. The murine strain invades the tunica albuginea testis of the guinea-pig on the fourth day, reaches a maximum abundance on the sixth and disappears between the tenth and twelfth days of infection. The louse-borne (European) strain does not invade the tunica of the guinea-pig but is found in the mesorchium. It appears on the fifth day and reaches a maximum abundance on the seventh day after the infection. On this day an occasional culture of the tunica is also positive. The rat strain has also been cultivated from the mesentery, peritoneum and pericardium. Infected testicles may be kept twenty-four hours at room temperature without injury to the rickettsias. Cultures incubated at from 28 to 30 C. survive and are infective up to from fifteen to twenty days. Subcultures retain their virulence for guinea-pigs.

FROM THE AUTHORS' SUMMARY.

PURIFIED VIRUS OF FOOT AND MOUTH DISEASE. I. A. GALLOWAY and W. J. ELFORD, Brit. J. Exper. Path. **17**:187, 1936.

It has been found possible to prepare a purified virus of foot and mouth disease in buffered phosphate solution at p_H 7.6 by repeated washing on collodion membranes of suitable porosity. The prepared virus gave a negative reaction in the test for protein (sulfosalicylic acid test) and had a potency equivalent to that of the stock filtrates. The stability of the purified virus in buffered phosphate solution over a period of about three days at 22 C. or one month at 0 C. compared very favorably with that of the unpurified virus. After much longer periods the loss in the titer of the purified virus was somewhat greater than that in the titer of the unpurified virus kept under the same conditions. Slight differences were observed in the stability of various batches of purified virus, doubtless attributable to varying degrees of purity below the least concentration of protein detectable by the sulfosalicylic acid test. The purified virus differed from the unpurified virus in (1) its relative instability in a digest broth medium, (2) its greater sensitiveness to the photodynamic action of methylthionine chloride (methylene blue) and (3) its greater susceptibility to the lethal action of certain concentrations of alcohol. The filtration behavior of the purified virus suspended in a buffered phosphate medium at p_H 7.6 was in every way similar to that of the unpurified virus under the same conditions. Tests on the survival of the purified virus at the critical p_H values 3, 6 and 7.6 showed that, as in the case of the unpurified virus, two zones of relative stability may be demonstrated, separated by a region in which the virus rapidly becomes inactivated. The number of experiments made was not sufficient to allow one to decide definitely whether or not any slight alteration in relative stability is shown by the washed virus, but certainly there appeared to be no pronounced displacement of the p_H zones of stability. The purified virus dried in vacuo remained infective for at least a week at —4 to 0 C.

FROM THE AUTHORS' SUMMARY.

THE PROPAGATION OF THE VIRUS OF INFECTIOUS ECTROMELIA OF MICE IN THE DEVELOPING EGG. F. M. BURNET and D. LUSH, J. Path. & Bact. **43**:105, 1936.

The virus of infectious ectromelia of mice can be propagated on the chorioallantoic membrane of the developing egg. The propagated virus produces the characteristic proliferative lesions. Formation of lesions and proliferation of the virus do not occur unless an incubation temperature slightly below the normal (39.5 C.) is used. Eggs used during the spring months show a distinctly reduced susceptibility to infection as compared with winter and summer eggs. During this period subinoculation with egg membrane material gave very poor lesions,

and passage experiments usually failed after a small number of generations. At other periods the virus was easily maintained by passage through the egg, giving typical lesions at each generation. Under suitable conditions the number of specific foci produced by diluted suspensions of the virus can be used as a measure of its patency, the results agreeing well with those obtained by titration in the mouse. Neutralization tests with mouse or rat immune serums can be carried out by the chorio-allantoic membrane technic. The results indicate a neutralizing effect of the same order as that determined by inoculation in the mouse. The histologic character of the lesions of the chorio-allantoic membrane is described; the two main features are the presence of typical cytoplasmic inclusions and the variation in the degree and type of protective reaction.

FROM THE AUTHORS' SUMMARY.

THE ASH CONTENT OF NORMAL AND PATHOLOGIC LARGE ARTERIES. W. WEPLER, Virchows Arch. f. path. Anat. **295**:547, 1935.

By slight modification of the Hackmann technic for the preparation of spodograms, Wepler obtained satisfactory preparations for a study of the distribution of inorganic salts in the arterial wall. In normal arteries the ash appears in the form of a regularly meshed network that corresponds to the elastic lamellae and muscle fibers of the media and intima. Calcification of the media begins in the third decade of life and earlier in the aorta than in the large arteries of the pelvis; it occurs in the form of finely granular deposits along the elastic lamellae. The material in primary medial calcification is chiefly calcium carbonate; in secondarily calcified areas it is largely calcium phosphate. Areas infiltrated by fats and lipoids are rich in sodium, which is probably present in the form of sodium soaps. Calcification of such areas results from the formation of calcium soaps, which are finally transformed into inorganic calcium salts. Atheromatous and lipoid areas contain less ash than normal areas. Beneath areas of pathologic intimal thickening the calcium content of the media is decreased.

O. T. SCHULTZ.

THE TIME OF APPEARANCE OF THE SPIROCHETES OF SYPHILIS IN REGIONAL LYMPH NODES. T. TANI, K. OGIUTI, H. HUTAKI and I. OYA, Zentralbl. f. Bakt. (Abt. 1) **134**:54, 1935.

Emulsions of spirochetes of syphilis were rubbed into scarified areas in the scrotal sacs of albino rabbits. The organisms were demonstrated in regional lymph nodes as early as five minutes later and in the blood within one hour. The spleen was spirochete-free for at least five days.

Local treatment of the infected scarified area was useless when washing with soap solution was tried, whereas a salve (presumably mercurial) protected two of four rabbits when applied immediately, but was without effect when used fifteen minutes after infection.

PAUL R. CANNON.

INTRACEREBRAL INJECTION OF VACCINE VIRUS IN WHITE MICE. G. BUCKUP, Zentralbl. f. Bakt. (Abt. 1) **134**:56, 1935.

Vaccine virus from three strains was grown in the allantoic membranes of hens' eggs (Goodpasture method) and injected intracerebrally into white mice. Typical meningo-encephalitis developed, and the animals died usually within from four to five days. This confirms the claims of Haagen that the white mouse can be used for the biologic demonstration of vaccine virus. Strains, however, which exert a marked effect in the rabbit skin may have but slight effect in the mouse brain.

PAUL R. CANNON.

Immunology

REINOCULATION WITH THE SPIROCHETES OF YAWS. T. B. TURNER, Am. J. Hyg. **23**:431, 1936.

In man, yaws confers immunity to reinfection. Resistance to auto-inoculation becomes manifest early in the course of the disease and obtains in most persons during the period in which active lesions are present. Immunity to reinoculation with heterologous strains of the spirochetes of yaws develops slowly during the course of the natural disease. Within the first three years reinoculation may give rise to a modified or an abortive attack of yaws, but after a period of ten years the majority of yaws-infected persons are refractory to reinoculation. An interruption of the normal course of the disease by treatment seems to retard the development of immunity. Syphilis also confers immunity to yaws, and this immunity is as great as, if not greater than, that conferred by yaws itself.

FROM THE AUTHOR'S SUMMARY.

RESEARCHES ON TETANUS. J. J. ABEL, E. A. EVANS JR. AND B. HAMPIL, Bull. Johns Hopkins Hosp. **59**:307, 1936.

Free tetanus toxin was not found in the blood of the horse, sheep or guinea-pig when these animals were inoculated with lethal doses subcutaneously or intramuscularly. However, the injection of supralethal amounts of toxin led to the recovery from the blood-lymph system of all but one lethal dose. Some of the viscera, e. g., the liver, showed profound changes resulting from the acute poisoning and also from repeated sublethal doses. The time of death was determined by the absolute amounts of toxin fixed by the various organs in the first few hours after the requisite threshold concentration in the tissue spaces had been attained or surpassed. No blood-brain barrier for the toxin was found. When the toxin was injected into the skin or muscles of highly susceptible animals, it was found almost in toto in the blood and lymph, showing that the various organs had exhausted their power to dispose of the excess, in contrast to their power to dispose of all of the toxin of a single lethal dose. In the refractory cold-blooded animals and fowls given large doses, the blood-lymph system for a considerable time accumulated even the smallest fraction of the injected lethal dose. The retention in the blood and lymph of sheep and horses of excessive quantities of the toxin beyond a lethal dose is explained by the inability of the cells of the various organs to take up additional amounts of toxin, although it was present in abundance in the tissue spaces. Different species of animals varied in respect to the time required for the disposal of supralethal quantities of toxin. When once the receptive substance of the voluntary muscles, the receptive cells of the central and autonomic nervous systems and the cells of the nonreactive organs fixed and absorbed the toxin, the excess of toxin present in the blood-lymph system was no longer significant in exerting a more poisonous effect. The toxin was recoverable from the blood and lymph and from incompletely washed tissues so long as the poison was in circulation, but it was not recoverable when fixed by the cells of the body. The cellular alterations of nonfatal tetanus were of reversible character; that is, the cells specifically affected by the toxin had the power not only to fix but to render innocuous and destroy sublethal doses, with return of normal function.

FREDERICK STENN.

LOCAL REACTIONS FROM INTRADERMAL INJECTION OF SMALL AMOUNTS OF ANTISERUM. B. C. SEEGAL and others, J. Allergy **7**:27, 1935.

Ten or twelve normal adults were given intracutaneous injections of 0.1 cc. of undiluted immune serum derived from four species of animals and of two globulin antibody solutions in their forearms. Local serum sickness, characterized by wheals, erythema and itching, occurred as follows:

1. Horse antipneumococcus type I serum produced a protracted severe sickness in eight of the twelve persons tested.

2. Sheep antistreptococcus haemolyticus serum produced a severe reaction in nine of the subjects and a slight reaction in one.

3. Rabbit antipneumococcus type I serum produced a slight local reaction in only one person.

4. Bovine antidiphtheria toxin serum produced a strong reaction in two and a moderate or slight reaction in six persons.

5. Equine antitetanus toxin globulin elicited only two moderate and three slight reactions.

6. Bovine antitetanus toxin globulin produced the mildest possible reaction in three subjects.

It is possible that such local reactions may be helpful in studying the tendency of therapeutic serums to produce serum sickness.

FROM THE AUTHORS' SUMMARY.

NEUTRALIZATION TESTS WITH SERUM OF CONVALESCENT OR IMMUNIZED ANIMALS AND VIRUSES OF SWINE AND HUMAN INFLUENZA. T. FRANCIS JR. and R. E. SHOPE, J. Exper. Med. **63**:645, 1936.

The viruses of human and swine influenza were regularly neutralized by the homologous immune serums. However, the serums of animals convalescent from infection with either virus possessed little, if any, neutralizing capacity for the heterologous virus. Hyperimmunization of animals against the swine virus tended to increase the neutralizing capacity of their serum for the human virus but in an inconstant fashion, whereas repeated inoculations with the human virus frequently resulted in serum with strong neutralizing activity against the swine virus. These observations serve to emphasize both the immunologic distinctiveness and the interrelationships of the viruses of swine and human influenza.

FROM THE AUTHORS' SUMMARY.

NEUTRALIZING ANTIBODIES FOR THE VIRUS OF HUMAN INFLUENZA IN THE SERUMS OF PERSONS OF DIFFERENT AGES. T. FRANCIS JR. and T. P. MAGILL, J. Exper. Med. **63**:655, 1936.

The results of mouse protection tests with 136 human serums and a strain of the virus of human influenza are described. After the first year of life, the serum of approximately half the persons tested contained sufficient antibody to furnish complete protection to mice. A much higher percentage of the serums obtained from persons recently convalescent from influenza exerted a completely protective effect. On the other hand, certain serums protected only partially under the conditions of the tests. The results have been compared with those obtained by Shope in tests done with the same serums against the virus of swine influenza. The possible epidemiological significance of the results is discussed.

FROM THE AUTHORS' SUMMARY.

NEUTRALIZING ANTIBODIES FOR THE VIRUS OF SWINE INFLUENZA IN THE SERUMS OF HUMAN BEINGS OF DIFFERENT AGES. R. E. SHOPE, J. Exper. Med. **63**:669, 1936.

Serums from a very high proportion of the human adults and new-born infants studied neutralized the virus of swine influenza; serums from children below the age of 12 years seldom exerted such an effect. The results of neutralization experiments with human serum and the virus of swine influenza have been compared with those from similar tests with the virus of human influenza, and it seems evident that the presence of antibodies neutralizing the virus of swine influenza cannot be deemed to be the result of repeated exposures to the current human virus. From the known history of swine influenza and the similarity of the etiologic virus to that obtained from man it seems likely that the virus of swine influenza is the

surviving prototype of the agent primarily responsible for the great human pandemic of 1918, as Laidlaw has already suggested. The presence in human serums of antibodies neutralizing the swine virus is believed to indicate a previous immunizing exposure to, or infection with, an influenzal virus of the 1918 type.

FROM THE AUTHOR'S SUMMARY.

THE PNEUMOCOCCIC TOXIN. A. F. COCA, J. Immunol. **30**:1, 1936.

Filtrates of cultures of Pneumococcus produced skin reactions and elevations of temperature as high as 105 F. in susceptible children. Convalescents were immune to the toxin of Pneumococcus type I. Skin tests seemed to suggest type-specificness of the toxin; on the other hand, neutralization tests and experiments with enzymes indicated the independence of the toxin and of the type-specific polysaccharides. The toxin of type II seemed qualitatively different from the toxins of the other types. Attempts to detoxify the filtrates with 40 cent solution of formaldehyde were not successful, because the antigenic properties and the toxicity were affected similarly. The antigenicity of the filtrates was dependent on the presence of carbon dioxide in the culture medium in concentrations of about from 15 to 20 per cent. The antigenicity was evidenced by the production of antitoxin in children. Serum from two convalescent patients neutralized the toxin of type I. These patients were recovering from pneumonia caused by type II.

I. DAVIDSOHN.

A SEROLOGIC COMPARISON OF THE PHOSPHOPROTEIN OF THE SERUM OF THE LAYING HEN AND THE VITELLIN OF THE EGG YOLK. R. R. ROEPKE and L. D. BUSHNELL, J. Immunol. **30**:109, 1936.

By producing precipitating immune serums in rabbits with serums of laying hens and of male birds and with vitellin (a protein of hen's egg yolk) a serologic confirmation was obtained of the previously clinically demonstrated similar phosphoprotein in the serum of the laying hen and in the egg yolk. It is possible that a small amount of such a phosphoprotein exists also in the serum of the male bird.

I. DAVIDSOHN.

STUDIES ON TETANUS TOXOID. F. G. JONES and J. M. MOSS, J. Immunol. **30**:115, 1936.

The superiority of alum-precipitated tetanus toxoid over the unprecipitated toxoid was again demonstrated on forty-five persons in whom two injections of the former produced a higher degree of immunity than one injection of the latter. Two of the most severe reactions occurred in persons given injections of the alum-precipitated toxoid. The authors advanced the explanation that the reactions were of an allergic nature, although both were delayed.

I. DAVIDSOHN.

THE DEVELOPMENT OF SENSITIZATION TO FOREIGN PROTEIN IN HUMAN BEINGS. J. R. MOTE and T. D. JONES, J. Immunol. **30**:149, 1936.

Changes in the sensitiveness of human beings to rabbit serum were observed over a period of nearly two years. The earliest manifestation of beginning sensitiveness was erythema, sometimes with edema, appearing twenty-four hours after the injection. It was similar to the bacterial type of hypersensitiveness. In the further course of increasing hypersensitiveness the immediate wheal and flare began to appear in response to the intracutaneous injection of rabbit serum. When the hypersensitiveness was decreasing the wheal and flare disappeared first, and the twenty-four hours delayed reaction followed. Very marked individual variations were observed, but most persons eventually became more or less sensitive after varying numbers of intracutaneous injections of rabbit serum.

I. DAVIDSOHN.

THE MECHANISM OF IMMUNITY TO RIFT VALLEY FEVER. G. M. FINDLAY, Brit. J. Exper. Path. **17**:89, 1936.

Evidence is presented to the effect that when an animal receives a mixture of the virus of Rift Valley fever and of immune serum its fate depends not only on the relative concentrations of virus and immune serum in the mixture but on the actual amount of the inoculum when that inoculum contains a virus-serum mixture that can be reactivated by dilution.

THE ANTIGENIC COMPOSITION OF THE VIRUS OF PSITTACOSIS. S. P. BEDSON, Brit. J. Exper. Path. **17**:109, 1936.

The complement-fixing power of a crude suspension of the virus of psittacosis depends on elementary bodies and more highly dispersed material, which is devoid of virulence, removed by Seitz filtration and thrown down to a certain extent by centrifugation at 10,000 revolutions per minute. The virus appears to contain two antigens, one resistant to boiling, the other rapidly destroyed at this temperature. Psittacosis antiserum contains antibodies to these two antigens, and union between them and their respective antigens in vitro can be demonstrated by complement fixation. The clear supernatant fluid which is left when the protein in a crude suspension of the virus is coagulated by heat contains a heat-stable antigen which gives an allergic skin reaction in the sensitized guinea-pig and a weak precipitin reaction with a specific serum but is of no use as an antigen in a complement-fixation test.

FROM THE AUTHOR'S SUMMARY.

NONSPECIFIC ALLERGY. PAUL BORDET, Ann. Inst. Pasteur **56**:325, 1936.

This paper is stated to represent the first of a series of studies of allergy. Various observations were compiled and experimental determinations were made of the peculiar hypersensitivity of tuberculous animals and persons to the protein of unrelated bacteria. The reactions were not always local as with typical Sanarelli or Shwartzman reactions but sometimes resulted in the death of animals. Death followed in a few hours in tuberculous guinea-pigs given injections of filtrates or killed cultures of Bacterium coli. These products were toxic, but the tuberculous animals were particularly hypersensitive. Injection of a suspension of colon bacilli previous to inoculation with tubercle bacilli did not prevent the allergic sensitivity, nor did previous injection of staphylococci intensify it. The nonspecific allergic reaction appeared to be intimately related to the presence of inflammatory tissue. That nonspecific allergy may be a factor in immunity through the prevention of dissemination from an area of infection, circumscribed because of such sensitivity, is given as a hypothesis for discussion in a subsequent paper.

M. S. MARSHALL.

AGGLUTINOGENS. G. BRUYNOGHE, Arch. internat. de méd. expér. **11**:529, 1936.

Of the various agglutinogens, only agglutinogen A maintained its antigenic power in the rabbit after it had been heated to 120 C. for half an hour. The heterogenic antigens and the antigen of Forssman from cells of type A were found not to be identical. Agglutinogens A and B were of protein and the heterogenic antigens as well as the supplementary agglutinogens M and N were of lipid nature. The injection of these lipids, obtained from pig serum, did not give rise to immuno-agglutinins M and N. The supplementary agglutinogens were not regularly excreted as were agglutinogens A and B. The studies on saliva were not convincing.

FREDERICK STENN.

ANTIGENIC PROPERTIES OF HORMONES. F. EICHBAUM and V. KINDERMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **86**:284, 1935.

An immune serum was produced by injecting into rabbits the anterior pituitary-like gonadotropic hormone from the urine of pregnant women. The reaction that

took place in the complement-fixation test was with an antigenic substance specific for the urine and closely associated with the hormone but not identical with it. No reaction took place with blood serum or with extracts of the anterior pituitary gland.

I. DAVIDSOHN.

ACTION OF PNEUMOCOCCIC ANTISERUMS. L. OELRICHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **86**:300, 1935.

Each of a series of mice was inoculated subcutaneously with 0.5 cc. of a potent type I pneumococcus antiserum and eighteen hours later was given an intravenous injection of living pneumococci in an amount that was equivalent to many fatal doses. The animals were killed after varying intervals. Sections were cut from different organs, and smears were prepared from the blood and organs. Within a few minutes after the bacteria were injected, their capsules swelled up, and they formed large agglutinated clumps, which were found mainly in the capillaries of the lungs and kidneys, where they underwent gradual but fairly rapid disintegration. The participation of the reticulo-endothelial cells within the spleen and liver was not noticeable at first, but after a while their phagocytic activity became apparent when they filled up with bacteria that reappeared in the circulation. The injection of dead pneumococci into passively immunized mice was followed by identical changes. Suspensions of organs of these animals failed to infect other mice even when the first animals were killed five minutes after they were inoculated with living pneumococci. Exposure of virulent pneumococci in vitro to immune serum or to the blood or tissue suspensions of passively immunized animals destroyed their ability to infect mice but did not interfere with their capacity to grow on culture mediums. The swelling of the capsule and clumping are part of the disintegration that takes place in fully passively immunized animals, while in actively immunized and in weakly passively immunized mice such a change was not observed, and the destruction and elimination of the bacteria came about from the activity of the fixed phagocytes, mainly those of the spleen and liver.

I. DAVIDSOHN.

IMMUNIZATION OF RABBITS WITH HUMAN SERUMS OF DIFFERENT BLOOD GROUPS. S. OLBRICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **86**:377, 1935.

Agglutinins specific for species but nonspecific for blood groups were produced by intravenous injections of serums from persons of different blood groups. The titers of these immune serums were quite low. No agglutinins were produced against the M and N factors. Absorption with erythrocytes of group O removed only agglutjnins against the O erythrocytes, while absorption with erythrocytes of groups A or B removed agglutinins against O erythrocytes in addition to the agglutinins against the type of erythrocytes used for adsorption. As a result of the absorption with the O erythrocytes, the serum reacted like an O isoserum, and after absorption with erythrocytes A, B or AB, the serum behaved like isoserum A or B or AB.

I. DAVIDSOHN.

Tumors

FOUR CASES OF EWING SARCOMA IN RIBS. H. BERGSTRAND, Am. J. Cancer **27**:26, 1936.

Four cases of Ewing sarcoma in ribs are described, all exactly similar. Each tumor originated from the posterior portion of a rib and grew, like a sponge, into the pleural cavity, pushing the lifted periosteum and the pleura before it. The greatest length of the tumor was along the rib. The surface was coarsely lobate. The involved rib presented a characteristic increase in density and volume, and spicules were formed. The latter occurred only on the inside of the rib, however, where the periosteum was elevated, and not on the outside, even though the tumor

tissue grew around the rib. The loosening of the periosteum began at the point of attachment of the intercostal muscles. One patient, treated by extirpation and roentgen irradiation of the sites of the tumor, is alive and well five and a half years after the operation, although two years after the operation metastases had occurred in the lungs. These disappeared under treatment with roentgen rays. Bergstrand believes that in practically all cases Ewing sarcoma is localized to a part of the skeleton in which ossification begins in the second month of fetal life. The first formation of the later ossifying blastema takes place at the same points. As the cells of Ewing sarcoma are very similar to the blastema cells, the conclusion might tentatively be drawn that the Ewing sarcoma is due to a disturbance in the formation of the skeleton at a very early stage of fetal life.

FROM THE AUTHOR'S SUMMARY.

MAINTENANCE OF HUMAN NORMAL CELLS AND TUMOR CELLS IN CONTINUOUS CULTURE. G. O. GEY and M. K. GEY, Am. J. Cancer **27**:45, 1936.

Normal human fibroblasts can be maintained under continuous cultivation for long periods of time and in mediums varying somewhat in composition. Several different types of human sarcoma cells can be maintained as pure strains for long periods of time under continuous cultivation by fairly simple procedures. There are certain differences and certain similarities in the cellular and cultural behavior of pure strains of human sarcoma cells cultured in cases given the same histologic diagnosis. The composition of the culture medium can be considerably varied and still allow maintenance of some pure strains of cells for considerable periods of time. A composite medium containing balanced salt solution, bovine embryo extract, human placental (fetal) serum and human plasma or human plasma with added chicken plasma can maintain human normal and human malignant tumor cells under continuous cultivation for long periods of time. The same composite medium is as suitable for the maintenance of rat malignant cells as for human malignant cells; a number of tumor cells of both kinds have been maintained for varying periods up to over four and a half years. The methods, mediums, technic and apparatus referred to in this article make possible the maintenance at the same time of some twenty to thirty different strains of cells by a small group of three or four workers. All of the procedures involved can be carried out by one person. The possibility of maintaining by continuous culture large quantities of human and animal malignant and normal cells for various experimental purposes is no longer a matter for conjecture, but has been proved.

FROM THE AUTHORS' SUMMARY.

THE REACTION OF MICE AND MOUSE TUMORS TO INJECTIONS OF BACTERIAL PRODUCTS. H. B. ANDERVONT, Am. J. Cancer **27**:77, 1936.

Studies on the toxicity of soluble bacterial products obtained from meningococci and colon bacilli have shown that the toxicity is enhanced for both tumor-bearing and normal mice if the animals are kept at 37 C. after the injection of the filtrates. Tumors growing within the skin of the mouse serve as excellent test objects in determining the activity of the filtrates. They may be used in carrying out quantitative experiments with the filtrates and present an opportunity for microscopic examination of the entire tumor following an injection of a filtrate. Both sarcoma 37 and sarcoma 180 show pronounced variation in their susceptibility to filtrates of cultures of Bacillus coli when grown cutaneously in different lines of pure strain or stock mice. These observations emphasize the importance of the strain of tumor-bearing mice used in experiments of this kind. Filtrates of cultures of B. coli failed to produce hemorrhage in practically all spontaneous carcinomas of the mammary glands of mice as well as in the first transplants of such tumors. However, these filtrates produced hemorrhage with regularity in primary 1, 2, 5, 6-dibenzanthracene tumors and in some instances brought about complete retrogression of the first transplants of such tumors.

FROM THE AUTHOR'S SUMMARY.

VARIATIONS IN SYSTOLIC BLOOD PRESSURE IN PATIENTS WITH RENAL TUMORS. C. G. MORLOCK and B. T. HORTON, Am. J. M. Sc. **191**:647, 1936.

This analysis shows a striking uniformity of the readings of systolic blood pressure for renal tumors of various histologic types. The ranges of blood pressure in cases of hypernephroma and of renal tumors of other types were practically identical. No consistent alteration in blood pressure occurred following removal of a tumor of either type. This was particularly significant in cases of hypernephroma, in which for both males and females as high an incidence of hypertension occurred after removal of the tumor as existed prior to operation. Scrutiny of the males with renal tumors other than hypernephroma leads one to think that they experienced diminution of arterial tension following operation, but since an exactly opposite situation existed with respect to the females, one can hardly attach significance to this. In conclusion, the investigators found no constant increase in the blood pressure of patients who were suffering from renal tumors. In particular they failed to substantiate the observation of previous investigators that a marked fall of an antecedent hypertension followed removal of a hypernephroma. Finally, it is suggested that this study offers clinical evidence that an epinephrine-like pressor substance is not produced by the hypernephromatous type of renal tumor.

FROM THE AUTHORS' SUMMARY.

GROWTH CURVES OF MALIGNANT AND NORMAL TISSUES OF THE RAT. R. SCHREK, Am. J. Path. **12**:525, 1936.

The growth of the Walker tumor and the rat embryo can be represented by linear curves. The growth rates of rat tumors, in vivo and in vitro, are in the same order of magnitude as the growth rate of rat embryonic tissue. After the embryonic stage the growth rate of normal tissues is markedly diminished. This decrease in growth rate is presumably due to the differentiation of the tissues. In contrast, there is no appreciable inhibition in the growth rate of malignant tumors like the Walker tumor. Transmissible rat tumors are characterized not by an abnormal proliferative capacity but by a lesser tendency to differentiate.

FROM THE AUTHOR'S SUMMARY.

PULMONARY ASBESTOSIS AND CARCINOMA. D. S. EGBERT and A. J. GEIGER, Am. Rev. Tuberc. **34**:143, 1936.

There is growing recognition of pulmonary asbestosis as a disease entity and industrial hazard. In twenty-eight fatal cases reviewed by Egbert (*Am. Rev. Tuberc.* **31**:25, 1935), pulmonary tuberculosis was a common complication. The authors report what seems to be the first case in which primary carcinoma was an associated condition, in a 41 year old factory hand. That the irritating effects of the inhaled asbestos particles may have been a significant factor in the development of the carcinoma seems plausible.

H. J. CORPER.

BUCCAL LEUKOPLAKIA. FRANCIS P. MCCARTHY, Arch. Dermat. & Syph. **34**:612, 1936.

McCarthy studied 316 patients with leukoplakia of the mouth and lips. The early stage of leukoplakia is characterized by a slightly sensitive red plaque, granular and sharply defined, which in a short time becomes slightly whitish gray. The second stage shows bluish white patches or plaques, quadrangular or irregularly polygonal, sharply demarcated, appearing to be pasted on the mucous membrane. Microscopic section shows hyperkeratosis and acanthosis with slight cellular reaction in the connective tissue. The fourth stage shows a keratotic lesion, indurated and leathery. Early malignancy is characterized by erosion, fissuring of the plaque or verrucous proliferation of the surface.

The causes of leukoplakia are given as faulty occlusion, chronic irritation, use of tobacco and syphilis. The condition is overwhelmingly prevalent in men, presumably because of the increased use of tobacco. It must be differentiated from chronic syphilitic mucous patches, lichen planus, lupus erythematosus and exudative processes in the mouth, such as erythema multiforme and pemphigus. Only in cases in which the condition involved the tongue could it definitely be determined that syphilis plays a rôle in the causation. Leukoplakia is to be considered definitely precancerous. Tobacco is by far the most important direct or indirect agent in the production of leukoplakia. Leukoplakia of the tongue due to syphilis originates in atrophy of the papillae due to endarteritis and subsequent irritation of the atrophic surface.

S. W. Becker.

The Daels-Biltris Transplantable Sarcoma of the Guinea-Pig. A. F. Watson, Brit. J. Exper. Path. **17**:122, 1936.

The behavior under laboratory conditions of the Daels-Biltris transplantable sarcoma of the guinea-pig is described and contrasted with that of the Murray liposarcoma. Its most interesting features are its vigorous growth, the frequency with which it metastasizes and, compared with rat and mouse tumors, its low content of reducing substances of a type similar to vitamin C.

From the Author's Summary.

The Chemical Reducing Capacity and Vitamin C Content of Transplantable Tumors of the Rat and Guinea-Pig. A. F. Watson, Brit. J. Exper. Path. **17**:124, 1936.

Progressive loss of reducing substances from normal and malignant tissues occurs when cevitamic acid (ascorbic acid) is withdrawn from the diet of guinea-pigs for from two to three weeks. These substances can usually be restored during this period by administering cevitamic acid in adequate amounts. Restoration to optimal values demands larger amounts of this acid than are sufficient to induce resumption of growth with typical repair in the teeth and ribs. The Jensen rat sarcoma dried in vacuo over sulfuric acid contains measurable amounts of vitamin C, which can be determined biologically. Two transplantable cavy tumors when dried under similar conditions showed only slight signs of activity of vitamin C, even when fed at much higher levels than the rat tumor. With these differences must be correlated the lower chemical reducing values of the cavy tumors compared with those of the rat. Within the limits of error of the tests, the antiscorbutic activity of dried Jensen rat sarcoma (as determined by feeding tests) approaches that demanded by its reducing activity if this is due to cevitamic acid. There is appreciable loss of reducing substances on desiccation of the tumor and the nature of these is not certain. Whether tumors contain in addition to cevitamic acid a hitherto unrecognized substance of high reducing activity remains undecided. The vitamin C reserves in the tissues of guinea-pigs on a scorbutic diet are exhausted more rapidly if the animals are supporting rapidly growing tumors. Like normal cells, tumor cells utilize vitamin C.

From the Author's Summary.

Blood Changes in Mice in Sarcoma. L. D. Parsons, J. Path. & Bact. **43**:1, 1936.

Sarcoma has been produced in mice by injection sub cutem of the water-soluble compound sodium 1, 2, 5, 6-dibenzanthacene-9, 10-endo-$\alpha\beta$-succinate. During the period of latency before tumor formation, abnormal cells appeared in the blood, but there was no large increase in the number of leukocytes. When the sarcoma appeared, a series of changes took place in the blood, namely (*a*) an increase in

the total number of leukocytes more or less proportional to the rate of growth of the tumor, (*b*) a rapid reversal of the normal relationship of lymphocytes and polymorphonuclears, the latter becoming more numerous than the former, (*c*) the appearance of increasing numbers of myeloid cells and (*d*) an alteration in the character of the polymorphonuclears, which were of large size and showed abnormal subdivision of the nucleus. Successive grafted generations derived from these primary sarcomas were accompanied by similar changes in the blood, which were roughly proportional to the rate of growth of the grafts. If the grafted tumor regressed, the total white cell count returned toward the normal level. Attempts to produce leukemia independently of a tumor by injection of blood or filtrates have hitherto been unsuccessful. The results suggest that there is present in the tumor an agent which may stimulate the formation of leukocytes of certain types. Certain systemic effects (enlargement and myeloid change in the spleen, infiltration of the liver around the portal tracts, cellular changes in the marrow, atrophy of the male genitalia) accompanied to a varying degree the growth of the grafted tumors. On five occasions a cell-free filtrate from each successive generation of one of the grafted sarcomas produced at the site of injection a sarcoma of the same or similar type. These five tumors all grew actively when grafted and have given "takes" in a very high percentage of the cases through many generations; they were propagated in this way much more readily than ordinary cellular grafts taken from the majority of the primary tumors. The growth of these strains of tumor derived from the filtrates was accompanied by changes in the blood and spleen similar to those associated with the growth of the primary tumors and cellular grafts. This similarity in the systemic effect suggests that the filtrates contained some specific factor derived from the parent tumor. FROM THE AUTHOR'S SUMMARY.

PSEUDOMYXOMATOUS EPITHELIAL GROWTH. M. A. ZACHARJEWSKAJA, Ztschr. f. Krebsforsch. **42**: 9, 1935.

The epithelial fragments in the mucus of appendical mucocele retain their reproductive properties and, if the mucocele bursts, are capable of dissemination throughout the peritoneal cavity, with secondary implantations resulting. The earlier stages of this are similar to conditions found in tissue cultures; later, with organization of the discharged mucus, there results a picture suggestive of chronic inflammation, which is added to by the discharge of fresh mucus from the implanted epithelial cells. Eventually, by a continuation of these processes, there may be formed a continuously growing pseudomyxoma, with epithelial structures occurring in the form of single cells, cell groups, laminae, cysts or relatively well developed gland tubules. H. E. EGGERS.

KAPOSI'S DISEASE AS A SYSTEMIC ANGIOMATOSIS. F. J. LANG and L. HASLHOFER, Ztschr. f. Krebsforsch. **42**:68, 1935.

Probably few apparently neoplastic diseases have been more variously interpreted than the entity commonly known as Kaposi's disease. It has been regarded as sarcoma of connective tissue origin, as malignant endothelial growth, malignant vascular growth, etc. There are described here the progressive lesions in two cases, and from them the writers conclude that the disease is primarily a vascular alteration, which takes the form of new capillary formation along with overgrowth of the endothelium of the capillaries and smaller vessels. Apparent internal metastases are regarded by them as the results of similar changes in those sites. In their opinion Kaposi's disease is a systemic hemangiomatosis, with the possibility, in the later stages, of perivascular connective tissue overgrowth and secondary vascular degeneration which may lead to eventual healing.

H. E. EGGERS.

SO-CALLED PLEURAL ENDOTHELIOMA. S. SCHEIDEGGER, Ztschr. f. Krebsforsch. **42**: 93, 1935.

In three cases of pleural neoplasm there were endothelium-like formations and peculiar caudate cells as well as masses of obvious epithelium. In a case of bronchiogenic carcinoma similar findings were present. Scheidegger is of the opinion that in none of these cases could the growth have originated in the pleural lining membrane. In a parallel case of tumor of the peritoneal lining, he observed definitely sarcomatous structures, and in a case of multicentric hepatic carcinoma, typical endothelium-like structures. He believes that all growths reported as endothelioma of the pleura are to be regarded as malignant, undifferentiated carcinoma, in part at least originating in epithelial rests. H. E. EGGERS.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Twenty-Fourth Annual Meeting, Memphis, Tenn., April 21-24, 1937

ALPHONSE R. DOCHEZ, *President*

SHIELDS WARREN, *Secretary*

Morning Session

Thursday, April 22

CORRELATION OF SERUM IRON, BONE MARROW AND BLOOD CELL CHANGES FOLLOWING SPECIFIC THERAPY IN THE MACROCYTIC ANEMIAS. CARL V. MOORE (by invitation) and CHARLES A. DOAN, Ohio State University.

The observation has been made that the nonhemoglobinous iron of the serum is higher than normal in the relapse phase of pernicious anemia. Beginning within twenty-four hours after the institution of adequate liver therapy, this iron fraction of the blood falls precipitously to a level that is below the normal range. The change thus precedes by three or four days the reticulocytosis that follows therapy.

Consequently, in order that the reequilibration in the serum iron could be more adequately understood with reference to the altered physiology of the erythropoietic tissue, serial supravital differential cell counts of sternal marrow, obtained by the puncture technic, were made for two patients with pernicious anemia both before and after the commencement of specific therapy. Blood for an analysis as to iron content was drawn within a few minutes after the sternal puncture was made.

Within twenty-four hours after the administration of liver began there was evident in the bone marrow a definite shift to the right in the level of the erythrocytic maturation. Megaloblasts were fewer, and mitotic figures were markedly increased. During the subsequent forty-eight hours a transition from a predominantly erythroblastic to a predominantly normoblastic marrow, with resultant discharge of reticulated red cells into the peripheral blood, was accomplished. The fall in serum iron closely paralleled the cytologic changes, reaching its greatest depression at the time of maximum delivery of erythrocytes from the bone marrow (the reticulocyte peak in the peripheral blood). These findings are interpreted as offering further evidence for the concept of serum iron as iron in transportation. It is considered that the decrease in serum iron occurred and persisted during these experiments for two reasons: (1) After the institution of liver therapy any increase in hemolytic activity promptly subsided, and (2) with the increase in synthesis of hemoglobin coincident with the reinstitution of normal maturation the iron being transported in the serum was withdrawn from the blood by the bone marrow and utilized at a more rapid rate than normal. The low serum iron under these conditions does not reflect inadequate storage of iron in the body depots.

In addition to hemoglobin iron and serum iron, a third form of blood iron has been described: "easily split-off iron." It is possibly, but not as yet certainly, of nonhemoglobinous origin. This third fraction remained at a fairly constant level during the changes described.

One of the two patients studied had addisonian pernicious anemia; the other had pernicious anemia of pregnancy, in which the disease had been produced primarily by a deficiency in a dietary extrinsic factor. The responses in cytologic

and serum iron in the two patients were identical and constitute further evidence that, from the standpoint of pathologic physiology, addisonian pernicious anemia and pernicious anemia of pregnancy reflect disturbances in essentially identical mechanisms.

VALUES OF PHOSPHATASE IN THE SERUM AND BILE OF DOGS WITH EXPERIMENTAL BILIARY FISTULAS AND IN DOGS WITH COMPLETE BILIARY OBSTRUCTION. SIEGFRIED THANNHAUSER (by invitation), STEPHEN MADDOCK, M. REICHEL (by invitation) and JEROME F. GRATTAN (by invitation), Boston City Hospital.

Experience with increasing and decreasing phosphatase activity and with the splitting of sphingomyelins by phosphatase suggested the possibility of activating the phosphatase of the blood serum in vitro. It was found that cevitamic acid (introduced as ascorbic acid) in appropriate concentrations would activate serum phosphatase. The activation is proportional to the concentration of cevitamic acid within certain limits and bears no relation to time. The optimum p_H is identical with that of normal phosphatase activity. As it was known that following complete biliary obstruction a marked rise in serum phosphatase occurred, the common bile duct was ligated in series of dogs, and studies were made of the serum phosphatase. It was found that after ligation the phosphatase of these serums rose from about 2 to about 100 Bodansky units in three weeks. These same serums were activated with 5 mg. of cevitamic acid per tenth cubic centimeter. The activity of all the serums rose to about 150 Bodansky units regardless of the initial value prior to activation, so that an almost uniform maximum activity was found after the addition of cevitamic acid, which was independent of the progressive increase in the concentration of phosphatase produced by biliary obstruction.

Dogs with total biliary fistulas were also studied, and it was found that the serum phosphatase rose to about ten times the normal levels. Cevitamic acid was effective in the serums of these dogs in the same manner as already described. The values of the phosphatase in the bile coming from the fistulas showed, in contrast to the values for that in the serum, almost complete activation.

In cats it was found that ligation of the common bile duct does not cause an appreciable rise in the blood phosphatase, but that the serums of these animals can be activated by cevitamic acid in the same manner as the serums of the dogs or human serums.

These experiments indicate that in complete biliary obstruction in the dog the increase in the value for circulating phosphatase is due to an increase in the activity of the phosphatase and not to an increase in its amount. They also indicate that in dogs with experimental biliary fistulas an increase in circulating phosphatase occurs without obstructive jaundice. It seems that the diagnostic value of an increase in serum phosphatase in certain pathologic conditions is limited.

CHRONIC HEPATOGENIC HYPOGLYCEMIA DUE TO ASCENDING CHOLANGIOLITIS, WITH RECOVERY FOLLOWING CHOLECYSTECTOMY. JEROME W. CONN (introduced by RAPHAEL ISAACS), University of Michigan Medical School.

The literature reveals no report of a case of chronic hepatogenic hypoglycemia due to ascending infectious hepatitis. An extensive study of the metabolism in such a case was made before and after the removal of the source of the ascending hepatitis. A remarkable recovery of the previously impaired glycogenic function of the liver was observed.

Before cholecystectomy frequent episodes of coma occurred spontaneously, with the blood sugar at such times ranging between 14 and 20 mg. per hundred cubic centimeters. Dextrose tolerance tests suggested that the hypoglycemia was due to deficient hepatic glycogenesis rather than to excessive production of endogenous insulin. Indirect calorimetry showed that while the blood sugar was dropping to hypoglycemic levels the oxidation of dextrose was normal. Numerous types of tests consistently revealed deficient hepatic function.

Exploratory laparotomy revealed a grossly normal pancreas. Biopsy of the liver showed active chronic cholangiolitis and biliary cirrhosis.

After cholecystectomy, gradual improvement occurred until virtual recovery was attained. No spontaneous hypoglycemic attack has occurred since operation. Dextrose tolerance tests are essentially normal. All tests show normal hepatic function. Indirect calorimetry still reveals normal oxidation of dextrose.

This study indicates that chronic infection of the biliary tract may so injure the glycogenic and glycogenolytic functions of the liver that severe spontaneous hypoglycemia results.

FACTORS INFLUENCING THE FATTY CONDITION IN THE DOG'S LIVER PRODUCED BY ALCOHOL AND A DIET RICH IN FAT. J. L. BOLLMAN, EUNICE FLOCK (by invitation) and F. C. MANN, the Mayo Foundation.

A diet rich in fat and poor in carbohydrate and protein causes the liver to become excessively fat within four or five weeks. If, to the animals receiving the fatty diet alcohol is administered twice daily in amounts which produce mild symptoms of intoxication, the liver becomes fatty within a few days. Animals receiving adequate carbohydrate and protein with the fatty diet are not influenced by similar quantities of alcohol. With the addition of dextrose in amounts sufficient to maintain normal amounts of glycogen in the liver to the diet of animals receiving fat and alcohol, less fat is deposited. The amount of dextrose required to inhibit the deposition of fat in the liver is considerably greater than the amount of pancreatic extract (alcohol soluble, ether and acetone insoluble) required to inhibit the deposition of fat under similar circumstances.

RÔLE OF ANOXIA OF THE TISSUES IN THE REACTION TO TUBERCULIN. JOHN S. HOWE (introduced by WILLIAM F. PETERSEN), University of Illinois.

Quantitative intradermal tuberculin tests with simultaneous use of two different dilutions of purified protein derivative (Long and Seibert) were made daily on a series of four patients with clinical tuberculosis and on two controls with no evidence of tuberculosis but with positive reactions to tuberculin. The two tests were made on symmetrical areas of the back, and the average diameter of the reactions was taken at twenty-four hours. The results were recorded after the method of von Groer: The product P equals the product of the average diameter times the negative logarithm of the concentration. (The shortest period of observation was one month and the longest eleven months.) Curves were plotted showing the day to day fluctuations of the level of allergy (or of sensitivity to tuberculin) in each patient, and these were compared with various clinical, chemical and serologic observations made daily. These curves revealed the following significant facts:

1. In every subject (patients and controls) there was marked daily variation in the reaction to the same standard dose of tuberculin, from 100 to 50 per cent of the most extensive reaction.

2. There was striking correlation between the quantitative variation in the tuberculin reaction and the state of tissue oxidation as measured roughly by the respiratory rate and more accurately by the time required for reduction of methylthionine chloride U.S.P.

3. In general, anoxia of tissues is characterized by decreased reaction to tuberculin.

4. Periods of anoxia are regularly followed by periods of increased oxidation of tissues associated with increased reaction to tuberculin.

5. In all subjects the trends of the curves of the tuberculin reaction taken at the same time were roughly parallel. This is in accord with the observations of Petersen, who has shown that the tissue oxidation-reduction phase is largely influenced by meteorologic environment, particularly by the dynamic infall of polar air, which produces vasoconstriction, resulting in tissue anoxia.

IMMUNOLOGIC AND BIOCHEMICAL PROPERTIES OF PROTEINS EXTRACTED FROM FUSOBACTERIA, ANAEROBIC STREPTOCOCCI AND BACTERIUM MELANINOGENICUM. CHARLES WEISS, Mount Zion Hospital, San Francisco.

In the course of an investigation as to the causation and pathogenesis of chronic pulmonary abscess, it seemed desirable to determine whether or not there exists a state of allergy to some of the anaerobic micro-organisms which are usually associated with this disease (fusobacteria, streptococci and Bacterium melaninogenicum).

Protein fractions were prepared by the method of Heidelberger and Kendall from four strains of fusobacteria belonging to types I, II and III of Slanetz and Rettger, two strains of streptococci (one a strict anaerobe similar to Streptococcus micros of Prévot and the other a facultative anaerobe similar to Streptococcus evolutus of Prévot) and two of Bact. melaninogenicum. For purposes of comparison, there were also included proteins extracted from two strains of Streptococcus haemolyticus (type A), one of Staphylococcus aureus and one of Pneumococcus (type III). Immune rabbit serums were prepared against these bacteria and reciprocal precipitin tests set up, with employment of a series of dilutions of the various proteins.

The tests showed that the fusobacteria, anaerobic streptococci and melaninogenicum bacteria contain proteins which are antigenically not only species specific but type specific. This observation is of special interest in view of the inability of Spaulding and Rettger to demonstrate type-specific carbohydrates in the fusobacteria.

INFLUENCE OF REYNALS-MCLEAN FACTOR VERSUS HOMOLOGOUS TUMOR MATERIAL ON THE BROWN-PEARCE RABBIT TUMOR. ALBERT E. CASEY, St. Louis University.

Stewart, Reynals and McLean have found large quantities of the powerful "spreading factor" in malignant tumor tissue, comparable with the amounts found in testicular tissue. It seemed worth while, therefore, to determine whether the marked enhancement of malignant disease induced with homologous tumor materials by Casey could possibly be due to this "spreading factor."

In a series of four experiments, balanced with adequate controls, each of thirty-three young adult male rabbits was given one injection of 0.4 cc. of bull or rabbit testicle extract (Reynals-McLean factor) in the right testicle, and two weeks later a saline emulsion of the Brown-Pearce rabbit tumor was injected into the left testicle. Thirty-five control rabbits, individually matched with the experimental series as to weight, breed, sex, age and physical condition, were inoculated with the same emulsion of the rabbit tumor but had received no previous injection. Between the control and the experimental series there was no significant difference as to the incidence and volume of the primary tumors, the incidence, the volume or the number of the metastases or the longevity and mortality after inoculation with the tumor.

This contrasted with the finding in a series of fourteen other experiments, also balanced with adequate controls, in which each of seventy-five young adult male rabbits was given one injection of 0.4 cc. of filtrable anaerobically refrigerated and preserved homologous tumor material in the right testicle and two weeks later was inoculated in the left testicle with a saline emulsion of the Brown-Pearce rabbit tumor. Eighty-five control rabbits, individually matched with the experimental series as to weight, breed, sex, age and physical condition, were inoculated with the same emulsion of the rabbit tumor but had received no previous injection. There was in every instance a significant enhancement in the course of the malignant disease in the experimental, as compared with the control, series; this was noted in the incidence and volume of the primary tumors, the incidence, volume and number of metastases, and the longevity and mortality after the inoculation with the tumor.

Furthermore neither the experimental nor the control series in the experiments with the Reynals-McLean factor differed as to the incidence or volume of the

primary tumors, or as to the incidence, the volume or the number of metastases, or as to the longevity or the mortality after the tumor inoculations from the control series in the experiments with the homologous material. In another experiment the spread of india ink in the flank was not increased over that found among control animals by the injection two weeks previously of 0.4 cc. of testicular extract. It is concluded that the effects on the course of malignant disease induced with the homologous material described by Casey cannot be explained by the possible activity of the Reynals-McLean factor, which such malignant tumor tissue might be expected to contain.

EFFECT OF ARTIFICIAL FEVER ON EXPERIMENTAL RABIES. F. D. McCREA (introduced by DOUGLAS H. SPRUNT) and HAROLD M. HORACK (introduced by DOUGLAS H. SPRUNT), Duke University.

Rabbits were inoculated intracerebrally with fixed rabies virus. Then, by exposure of the animals to radiant heat, their body temperatures were elevated and maintained between 41.2 and 41.8 C. (106.1 and 107.2 F.). Unheated controls were checked against each heated group.

The experimental animals were divided into five groups according to the number of hours of exposure to heat and the time of exposure as follows: (1) seven or twelve hours the day of inoculation; (2) six, seven or twelve hours twenty-four hours after inoculation; (3) seven hours the same day and seven hours forty-eight hours after inoculation; (4) twelve hours the same day and twelve hours forty-eight hours after inoculation; (5) twelve hours the same day and twelve hours forty-eight hours after inoculation.

The first four groups received 0.2 cc. of virus diluted 1:200; the fifth group, 0.2 cc. of virus diluted 1:100.

All controls and heated animals in groups 1, 2 and 3 died of rabies within from seven to fourteen days. In group 4 all nine controls died of rabies; of the fifteen heated animals, three died during the heating period and five of causes other than rabies; seven survived and by Feb. 13, 1937, had outlived their corresponding controls sixty-two, seventy-six, ninety, one hundred and two and two hundred and twenty days. Two controls and four heated animals comprising group 5 died of rabies. Whether this is significant we do not know.

Forty white rats were inoculated intracerebrally with 0.05 cc. of virus diluted 1:50 and on up to 1:400. In all the rats symptoms typical of rabies developed, followed by death. Blood serum from two surviving rabbits was mixed with virus, making dilutions of the latter varying from 1:50 to 1:400; these were incubated two hours at 37.5 C. and inoculated intracerebrally into thirty-six rats. All died with symptoms typical of rabies. Like dilutions were inoculated intracerebrally into eight rabbits, which also died with paralyses typical of rabies. Serum from surviving rabbits apparently has no protective action and seems to transfer no immunity.

NONTRANSMISSIBILITY IN UTERO OF TRICHINOSIS IN THE RAT. LLOYD CATRON (introduced by CARL V. WELLER), University of Michigan.

Each of twenty-one young adult white rats (weighing from 180 to 280 Gm.) was fed by stomach tube approximately 15 larvae of Trichinella spiralis per gram of body weight shortly before or during pregnancy. The time of feeding varied from one day before to fifteen days after copulation; the period of gestation was from twenty-one to twenty-three days. The placentas and tissues of the nine fetuses of one mother near term and the tissues of seven new-born rats (from six mothers) were examined thoroughly for larvae in fresh as well as in fixed and stained preparations. From sixty to seventy days after their birth, one male and one female from each of seventeen litters of trichinous mothers were skinned, eviscerated and digested in artificial gastric juice. No evidence of intra-uterine transmission of trichinosis in the rat was obtained. Further experiments are in progress.

Afternoon Session

Thursday, April 22

FOREIGN BODY REACTION OF THE MYOCARDIUM, WITH SPECIAL REFERENCE TO THE FORMATION OF GIANT CELLS AND SO-CALLED MYOCYTES. OTTO SAPHIR, Michael Reese Hospital, Chicago.

To determine whether or not foreign body giant cells in the myocardium resemble the multinucleated cells seen in the Aschoff nodules and to determine also, perhaps, the origin of the so-called myocytes, the hearts of a number of dogs were sutured with silk ligatures to the diaphragms. This was done also to compare the foreign body reaction in the myocardium with that of the diaphragm (transverse striated muscle). The optimal time for the formation of giant cells in the myocardium was found to be from two to three weeks. The giant cells in the myocardium were similar to those in the diaphragm and did not resemble the multinucleated cells of Aschoff bodies. To determine the origin of the myocytes, intravital dyes were used. It was found that the cells described as myocytes took up dye granules. In none of the sections was there seen an attempt toward regeneration of muscle fibers. In several instances cultures were made of the cells which were found surrounding the sutures. Invariably macrophages and connective tissue cells were grown, but never muscle cells or fibers. It seems clear that so-called myocytes do not exist in the heart and that the cells described as myocytes are simply altered endothelial leukocytes and macrophages.

TRANSFERS OF LIVING LEUKOCYTES. BERNHARD STEINBERG, Toledo Hospital, Toledo, Ohio.

Leukocytic exudates were produced in the peritoneal and pleural cavities of animals in from three to forty-eight hours and transferred to other animals of the same or other species with a preexisting infection. Bacterial and leukocytic counts and a study of the inflammatory exudate were made to determine the preservation of the phagocytic activity of leukocytes transferred to animals of the same or other species. The ability of such transferred leukocytes to overcome infections, otherwise fatal, was ascertained. The influence of the following factors on the phagocytosis displayed by transferred leukocytes and on the survival of the infected animal was determined: (1) the leukocytic extract, (2) the pleural or the peritoneal serum exudate, (3) the age of the untreated leukocytes, (4) the relation of the number of leukocytes to the severity of the infection, (5) the age of leukocytes in the body and (6) the duration of the infection. The terms "homologous passive leukocytoperosis" and "heterologous passive leukocytoperosis" are suggested for such transfers of leukocytes from one animal to another of the same or a different species.

CHEMOTROPISM IN LEUKOCYTES: THE SOURCE OF THE ATTRACTIVE SUBSTANCE. MORTON MCCUTCHEON and HAROLD M. DIXON, University of Pennsylvania School of Medicine.

For many years it has been a question among pathologists whether polymorphonuclear leukocytes are attracted to inflamed tissues by substances elaborated by bacteria or by products of cell and tissue breakdown. We studied this question by means of an experiment in vitro so devised that during a part of the experiment the center of attraction included bacteria alone, while later in the experiment the center of attraction included both bacteria and cells phagocytizing them. The intensity of the chemotropic response of leukocytes was determined under these two conditions.

PARENTERAL PROTEIN IN PNEUMONIAS. CLYDE BROOKS, Louisiana State University Medical Center, New Orleans.

In 1,200 cases of pneumonia, including all forms and patients of all ages, the best routine treatment of the medical center was used, including a hygienic regimen, nursing, medication, and surgical intervention when indicated.

One half of the patients were used as controls; the other half were treated by the parenteral administration of deuteroproteose.

The results show that among the controls the death rate was 39 per cent for lobar pneumonia and 32 per cent for bronchopneumonia. The death rate in the cases in which treatment was by deuteroproteose was 10 per cent for lobar pneumonia and 9 per cent for bronchopneumonia.

The results are encouraging, if not conclusive. They indicate that the parenteral use of deuteroproteose reduces the death rates for both lobar and bronchial pneumonia.

EXPERIMENTAL PULMONARY EDEMA AND BRONCHOPNEUMONIA. PAUL R. CANNON and THEODORE E. WALSH (by invitation), University of Chicago.

We have shown in earlier papers that bacterial suspensions introduced into the nostrils of normal rabbits quickly enter the lungs by way of the trachea and bronchi. This fact affords a simple and nontraumatic method for the study of the relationship between infection of the upper and infection of the lower respiratory passages. Furthermore, it enables one to observe the pulmonary effects of certain therapeutic agents which are frequently introduced intranasally into human beings.

We demonstrated in the experiments described the ease with which pulmonary edema and bronchopneumonia may be produced in normal rabbits merely by intranasal instillation of cultures of various bacteria. The cellular reactions and the varying effects are described. The pulmonary edema is regional in type and is due, presumably, to toxic injury of the capillary walls.

The intranasal instillation into healthy rabbits of certain commonly used therapeutic agents is also frequently followed by pulmonary edema and bronchopneumonia. If these agents are dissolved in light oil, as is the case with many of the so-called nasal sprays now in vogue, pulmonary edema and lipoid pneumonia result.

These experiments are a part of a study of the general problem of pulmonary edema and bronchopneumonia. They also demonstrate some of the potential dangers attending the indiscriminate use of certain therapeutic substances commonly employed in the treatment of infections of the upper respiratory tract.

ANTIBODIES FOR TRICHOMONAS FOETUS. PHYLLIS M. NELSON (introduced by A. L. TATUM), University of Wisconsin.

The microscopic demonstration by Witte of agglutinins for Trichomonas foetus in the serums of infected guinea-pigs was confirmed.

Tested by a microscopic method, the serums of rabbits given repeated intravenous injections of suspended live and formaldehydized parasites from three bacteria-free strains of T. foetus (isolated by Glaser and Coria) showed agglutinin in high titers. All the serums had been negative for agglutinins before immunization. The serum of a rabbit given repeated intravenous injections of suspended formaldehydized parasites from a strain of Trichomonas parva (mixed with bacteria) and the serums of two rabbits infected with Trypanosoma hippicum and Spirochaeta pallida, respectively, also gave negative results in tests for agglutination of T. foetus. The curve for the rise and fall of the agglutinin with time is similar to those for bacterial agglutinins. There is some indication of strain specificity. There seems to be no constant difference between the live and the formaldehydized parasites either as immunization or as test antigens.

Precipitins for T. foetus, absent from the serum of a rabbit before immunization, were present after immunization. Precipitinogen was demonstrated in the

vaginal washings of an infected rabbit in which there were many trichomonads, but not in the vaginal washings of two rabbits with few and no trichomonads, respectively.

EXPERIMENTAL PATHOLOGIC STUDY OF LYMPHOGRANULOMA VENEREUM. RIGNEY D'AUNOY and EMMERICH VON HAAM (by invitation), Louisiana State University Medical Center, New Orleans.

Lymphogranuloma venereum in man is characterized by the great variety of manifestations, which are partly determined by such factors as the sex of the patient, the portal of entry of the virus and its virulence. Because of these factors and a varying racial susceptibility to the disease, a much greater variety of lesions can be produced in experimental animals, and each of the lesions can be regarded as a potential manifestation of the disease in man. The work reported represents a study of all types of experimental lesions produced thus far in various laboratory animals which have proved susceptible to the virus.

SENSITIVITY OF CHROMOSOMES TO ROENTGEN RAYS IN RELATION TO TEMPERATURE AND p_H. ALFRED MARSHAK (introduced by SHIELDS WARREN), New England Deaconess Hospital, Boston.

Previous studies have shown that chromosomes are most sensitive to roentgen rays at the onset of the prophase, when division of the chromonemes occurs. Cells irradiated in this stage reach the midanaphase in from two and a half to three and a half hours after irradiation. Plotting the logarithm of the percentage of normal cells (those showing no abnormalities of the chromosomes) at three hours after irradiation as a function of the dose gives a straight line. The sensitivity of chromosomes to roentgen rays therefore follows a simple exponential law, $\frac{N}{No} = e^{-k}$, in which $\frac{N}{No}$ is the percentage of normal cells and k the slope of the curve to the e base; k has different values for different organisms, its magnitude varying directly as the total length (l) of the chromonemes of the chromosome complement of the cell. The same law holds for mouse sarcoma CR 180, rat carcinoma Walker 256, the broad bean, the pea, the onion and the tomato, which give a wide range of values of l.

From the values of k and l the diameter of the portion of the chromoneme which is sensitive to roentgen rays may be calculated. In the three organisms in which l has been determined accurately, this diameter (d) is 10×10^{-8} cm. This corresponds exactly with the dimension obtained from roentgen ray diffraction studies, which has been identified with the average separation of polypeptide chains in a variety of proteins. It also corresponds to the diameter of a protamine molecule. Calculations from published data on the frequency of roentgen ray-induced mutations in Drosophila give good agreement of both the length and the diameter of the clupeine molecule with that obtained for the sensitive volume of the gene if it is assumed that the latter has the diameter of 10×10^{-8} cm. as its short dimension.

Numerous experiments, beginning with those of Crowther, show that only positively charged colloids are precipitated and that proteins are agglomerated only when at the iso-electric point or on the acid side. From morphologic evidence alone it seems quite likely that the chromosomal abnormalities observed are produced by a process analogous to that of protein agglomeration. On the assumption that the milieu about the chromosomes has a p_H of 7.5, the only proteins likely to be positively charged are the protamines and histons, which have iso-electric points at about p_H 9 to 10.

To test this point, onion seedlings were immersed for half an hour in solutions of ammonium hydroxide in distilled water in concentrations ranging from 0.00001 normal to 0.01 normal and then irradiated. At the latter concentration abnormalities are observed which may be attributed directly to the action of the ammonia

and not to that of roentgen rays. In the range from 0.008 to 0.001 normal the cells and chromosomes show little if any direct effect of the ammonia. However, after irradiation the ratio $\frac{N}{No}$ is raised from the control value of from 0.50 to 0.55 to from 0.78 to 0.83. In other words, when the intracellular p_H is shifted above the iso-electric point of protamines and histons the frequency of abnormalities is decreased, which is in accordance with expectations on the basis of the aforementioned hypothesis. Treatment with a series of mixtures of carbon dioxide and air (carbon dioxide concentrations, 10, 20, 40, 60 and 80 per cent) showed no change in the $\frac{N}{No}$ ratio, indicating that if other proteins in the chromosome are capable of reacting to produce abnormalities their iso-electric points must be lower than that corresponding to the 80 per cent carbon dioxide mixture.

The flocculation of egg albumin after irradiation with ultraviolet rays has been considered a two step process: The first step is the denaturation of the protein by the radiation; the second, which involves interaction of the denatured molecule with water, has a temperature coefficient. To determine whether analogous behavior might be obtained with chromosomes, seedlings were incubated at 37.5 C. and fixed at various intervals. The frequency of mitoses dropped to zero within three hours, but by the end of seven hours at the same temperature mitoses began to appear again in appreciable numbers. There were no chromosomal abnormalities or other nuclear changes. When the seedlings were treated with 80 roentgens and then incubated at 37.5 C., the frequency of mitoses dropped steadily to a minimum at two hours after irradiation and then increased again. There was no complete disappearance of mitoses. Instead of the usual steady decrease in $\frac{N}{No}$ for chromosomal abnormalities between one-half hour and three hours after radiation, the curve showed two minima: One occurred at one hour, with $\frac{N}{No}$ equaling 0.75; the curve then rose to 0.91 at one and a half hours and then fell to 0.81, 0.59 and 0.53 (the second minimum) at two, two and a half and three hours, respectively. The value at three hours was no different from that for the controls at 23 C. Thus, though an increase in temperature of this magnitude produces disturbances in the mitotic process, it does not increase the number of abnormalities of the chromosomes. This suggests that, as far as roentgen ray-induced abnormalities are concerned, chromosomes do not behave as though albumins were involved.

STUDIES IN CARCINOGENESIS: VII. COMPOUNDS RELATED TO BENZPYRENE. M. J. SHEAR, Harvard University Medical School.

In collaborative studies of the carcinogenic activity of polycyclic compounds, compounds related to 3,4-benzpyrene were examined. These compounds, synthesized by Prof. L. F. Fieser and his colleagues of the division of chemistry of Harvard University, were all introduced subcutaneously into mice of a pure strain.

The 1′,2′-dihydro derivative of 4′-methyl-3,4-benzpyrene produced tumors in a few mice. In several cases the injected hydrocarbon was found inside the tumors at autopsy. Two of the tumors were successfully transplanted.

While 4′-keto-1′,2′,3′,4′-tetrahydro-3,4-benzpyrene was inactive, a preparation of the tetrahydro derivative (which possibly consisted of a mixture of hydro compounds) was found to be carcinogenic. At autopsy the injected hydrocarbon was seen in the interiors of several of the tumors.

The production of tumors with hydrogenated derivatives of aromatic carcinogens raises the question whether the body is capable of dehydrogenating such compounds.

No sarcomas were produced at the site of injection by 4′-hydroxy-3,4-benzpyrene or by 4′-methoxy-3,4-benzpyrene. Negative results at the site of injection were also obtained with the 1′,2′-dihydro derivative of 3,4-benzpyrene-3′,4′-dicarboxylic anhydride and with 3,4-benzpyrene-3′,4′-dicarboxylic anhydride. The disodium and dipotassium salts of 3,4-benzpyrene-3′,4′-dicarboxylic anhydride were both toxic.

Tests with 9-methyl-1,2-benzanthracene and with 5,9-dimethyl-1,2-benzanthracene are still in progress. These compounds, synthesized by Dr. M. S. Newman

of the department of chemistry of the Ohio State University, bear a structural resemblance to 3,4-benzpyrene, for the latter compound may be regarded as a 1,2-benzanthracene derivative with a carbon substituent at the 9-position. The 9-methyl compound gave negative results after ten weeks. The 5,9-dimethyl compound, however, produced after one month the same type of ulceration that had previously been obtained with rapidly acting carcinogens. The first tumor was obtained in ten weeks.

STAGES IN THE DEVELOPMENT OF THE MONOCYTE. RAPHAEL ISAACS, Simpson Memorial Institute, Ann Arbor, Mich.

[The article was read by title.]

Late in the monocytoblast stage, large granules, staining bluish red, appear in the cytoplasm and also are evident overlying the nucleus. The diameter of the cell at this stage is from 15 to 16.5 microns, and there is a round or slightly oval nucleus from 12 to 12.5 microns in diameter (measured in stained films). Maturation beyond this stage is accompanied by a decrease in the size and in the basophilia of the granules by an increase in the diameter of the cell. The granules persist as very fine eosinophilic granules, which are found in the adult monocyte. The cells in the stage in which the granules are somewhat basophilic have been erroneously classified as promyelocytes, myelocytes or metamyelocytes. The monocytes at this stage differ cytologically in many points from the cells with which they are confused. The monocytes are found in normal bone marrow, indicating that this location is a normal site for their development. In monocytic leukemia the crowding out of immature myeloid cells into the blood stream gives rise to the occasional misinterpretation as to the type of leukemia.

PATHOLOGICAL SOCIETY OF PHILADELPHIA

Nov. 12, 1936

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A FOREIGN BODY OF LONG STANDING IN THE BILIARY DUCTS. NED RAKER.

A white woman 33 years old was admitted to the Jefferson Medical College Hospital, May 11, 1934, with a complaint of painless jaundice occurring at increasingly frequent intervals over a period of eight years. The attacks of jaundice followed cholecystectomy in 1926 for attacks of epigastric colicky pain, nausea and vomiting of one year's duration. The operation was done at a distant hospital, and details concerning it could not be learned, but drainage was instituted for seven weeks after the operation, and then appendectomy was done for acute appendicitis and drainage instituted for four weeks. The attacks of jaundice began three months after discharge from that hospital.

On examination there were marked jaundice, tenderness in the upper right quadrant of the abdomen, enlargement of the liver and spleen, marked secondary anemia and retention of from 40 to 100 per cent of bromsulfalein dye in various tests. Obstructive jaundice was diagnosed. Roentgen examination of the abdomen revealed a metallic foreign body in the upper right quadrant, beneath the liver and in the region of the biliary tract. Therapy was begun with a view to preparing the patient for operation. After temporary slight improvement she had a profuse hemorrhage from the gastro-intestinal tract, failed rapidly and died on July 5, 1934, no operation having been performed.

At autopsy, the gallbladder was absent, and the common bile duct was much distorted and adherent to the first portion of the duodenum, into which it opened

through a fistula. All the biliary passages were dilated. Blocking the right hepatic duct and extending into the common duct was a metallic foreign body 4.2 cm. in length, crusted with a greenish deposit. The distal end of this body lay opposite the opening of the duodenal fistula in the common duct. On removing the concretion, the foreign body was found to be a broken blade of a hemostat, with the rough end opposite the duodenal fistulous opening. There was evidence of a massive hemorrhage in the gastro-intestinal tract and in the wall of the intestine. The liver was enlarged, the surface roughened and granular, and, on section, the fibrous tissue was found increased and the bile ducts markedly dilated. The source and the method of entrance of the foreign body into the biliary tract could not be definitely established.

METASTATIC CALCIFICATION ASSOCIATED WITH CARCINOMA OF THE BREAST. JOHN W. EGOVILLE.

A white woman 30 years of age was admitted to the Rhode Island Hospital with a history of increasing weakness and severe shooting pains over the shoulders, back and lumbar regions. Roentgen studies revealed multiple circumscribed areas of rarefaction in most of the long bones of the body, which suggested multiple myeloma, but a biopsy of a nodule in one of the ribs revealed an adenocarcinoma secondary to a lump in the right breast. On repeated examinations the blood urea averaged approximately 40 mg., serum calcium 18 mg. and serum phosphate 5 mg. per hundred cubic centimeters.

At autopsy, one hour after death, a walnut-sized nodule was found in the medial aspect of the right breast with extensive metastasis to the bones and involvement of all of the para-aortic lymph nodes. Most interesting were the calcium incrustations which covered the entire endocardial surface of the left auricle as an egg-shell-like material, excepting only a narrow longitudinal sulcus. The valves were normal. No gross calcium deposits were found in the kidneys, stomach and lungs. The parathyroid bodies were neither enlarged nor increased in number.

Microscopically, sections of the auricle presented a widened and hyalinized subendocardial layer diffusely impregnated with calcium granules. Beneath this there was a scattering of round cells about the fragmented elastic fibers. In sections of the lungs there was calcium deposition in the alveolar septums, in the submucosa of the bronchioles and beneath the intima of the pulmonary veins. Calcium granules were seen in the glomerular tufts, also, and calcified casts lay in the convoluted tubules.

Eighty-one years ago Virchow coined the term "metastatic calcification" for the process represented in the calcific deposits which he found in the kidneys, stomach and lungs in several cases of primary and secondary tumors of bone. This condition is associated with diseases in which pronounced destruction of bone occurs, the calcium being deposited chiefly in organs through which acid is eliminated, but its deposition is not preceded by cellular destruction. Evidence points to the cortex of the long bones as the source of calcium in this condition, associated hypercalcemia being due to the rapid dissolution of the bone by the tumor nodules, and the condition is not necessarily accompanied by hyperparathyroid activity.

HISTOGENESIS OF THE PLASMOCYTE. B. R. REITER and R. P. CUSTER.

Theories regarding the origin and nature of the plasmocyte are numerous, and no one group of data is completely satisfactory. Perhaps the most widely accepted belief at the present time is that the plasmocyte is closely linked with the lymphocyte, i. e., it is an offspring of this developmental series, because (1) there are certain morphologic resemblances between these cells, (2) they occur together in chronic inflammatory exudates, and (3) Maximow reported the appearance of plasmocytes in cultures of lymphoid tissue. These cultures, however, were made from chopped rabbit lymph nodes, necessarily containing cells

other than lymphocytes; cells of the reticulo-endothelial system can be identified in photomicrographs illustrating his work.

We believe that the plasmocyte develops from the indifferent mesenchymal reticulum (reticulo-endothelial system) in common with the other cells of the hemocytopoietic system, and has an individual developmental series through a clearly identifiable plasmoblast stage. This belief is based on histologic study of the cell as it occurs in relatively normal states (chronic inflammation) and in abnormal conditions arising through unrestricted proliferation (multiple myeloma, plasma cell leukemia). We are aware of the criticism usually offered regarding conclusions about an active, dynamic process through study of static material (fixed tissues); this was borne in mind constantly while our observations were being made, but transition forms were so clearcut that we were forced to make the interpretations presented here. Tissues were fixed both in 4 per cent formaldehyde and in Helly's fluid embedded in paraffin and stained with hematoxylin-eosin, with a modification of Maximow's azure II eosin with Giemsa's stain.

Nuclear Changes.—From the characteristic ovoid vesicular nucleus of the reticulum cell, with its delicate perinuclear membrane and fine chromatin network, the following sequential alterations occur: The nucleus becomes round and smaller, the perinuclear membrane thicker, and the chromatin granules very much coarser, the latter coming to lie just within the membrane so that the typical cartwheel pattern is formed. From a central position the nucleus becomes eccentric. Nucleoli tend to disappear as the adult stage is approached.

Cytoplasmic Changes.—The light blue-gray cytoplasm of the resting reticulum cell becomes more and more deeply basophilic, and there is transition from the fixed stellate form to a free round or ovoid state. Progressive reduction in size occurs, and a pallid, faintly acidophilic zone appears in the center, adjacent to the nucleus (perinuclear halo). The stage at which a given cell breaks off from its fixed position varies greatly; occasionally, except for the stellate shape, the characters of a fairly mature cell are present (this is not a packing artefact).

The developmental process is most strikingly shown in the liver in plasma cell leukemia, in which there is autochthonous formation of plasmocytes through metaplasia of Kupffer cells. The presence of more (and younger) plasmocytes in sections of the radicles of the hepatic veins than in vessels of viscera not showing the metaplastic foci tends to support the thought that these cells were formed in the liver in this instance.

In many instances there was no association between foci of plasmocyte proliferation and lymphoid tissue, and in no instance were we able to demonstrate transition forms between these two cell types.

GLOMAL TUMORS. HENRY E. RADASCH.

This article will be published in full in a later issue of the ARCHIVES.

A CRITICISM OF LABORATORY ROUTINE IN MODERN INSTITUTIONS OF PATHOLOGY. N. CHANDLER FOOT (by invitation).

In journeying through American and Canadian laboratories one seldom sees routine microscopic sections prepared in any way other than by the application of the hematoxylin-eosin technic to tissues fixed in formaldehyde solution. While this method is reliable, it is very limited in its scope. Some hints on the use of more modern methods which may prove helpful are given here.

Fixation.—It is important to have thin slabs of tissue and to fix them in a copious amount of fixing fluid. The practice of throwing whole masses of tissue into a small amount of fixative should be discouraged. Four millimeters is the theoretical maximum thickness of the ideal slab. The choice of a fixative is simple if one knows what one wishes to do with the fixed material. Experiments on normal nerve, fixed in four groups of fixative and impregnated in an identical manner with silver, show four groups of histologic pictures corresponding to: (1) the alcoholic group, (2) the solution of formaldehyde group, (3) the

chromate group and (4) the trinitrophenol group. This illustrates how staining results may be influenced by varying the fixing fluid. Formaldehyde-alcohol (strong solution of formaldehyde, 10 parts, and 95 per cent alcohol, 90) is a much better fixing medium than is generally supposed; it acts rapidly and causes comparatively little shrinkage. Material that promises difficulty in diagnosis should always be fixed in several fluids, so that aniline dyes, silver solutions and the like may be most advantageously used. Secondary "zenkerization" or mordanting in chromates, like secondary bromuration of nerve tissue, is never as good as primary chromatization or bromuration.

Embedding.—Dioxane (diethylene dioxide) dehydrates tissue rapidly and, as it is miscible with water or paraffin, is an ideal dehydrating and clearing agent. It leaves the tissue soft and pliable, which is an added advantage in subsequent sectioning.

To avoid shredding and crumbling of paraffin, it is well to prepare a stock of the following mixture to be used for embedding: Four or five parts of crude rubber (20 Gm.) melted and stirred while hot into 100 Gm. of paraffin (melting point 56 C. in winter and 58 C. in summer), plus 100 parts of paraffin and 1 part of yellow wax. This mixture is very cohesive, keeps its consistence well and does not tear or crumble as easily as pure paraffin. An electric refrigerator is a great help in the laboratory.

Sectioning.—The Mallory method of using a Minor microtome for cutting ribbons and laying these on hot water in a pyrex pie dish over an electrical hotplate is the best for mass production. The sections are cut apart with a hot knife while the ribbon floats on the surface. The single section method, formerly in use, is obsolete and should be used only in a technic employing pyroxylin (celloidin). In making frozen sections it is best to use the Mallory and Wright pyroxylin (celloidin) method on tissue fixed by bringing it to a boil in neutral solution of formaldehyde. This method takes a little longer, but the results are permanent and the sections when properly mounted and stained are as good as paraffin sections. The Terry method of cutting thin, translucent slabs of tissue with a razor and staining the upper surface is very valuable when speed is indispensable or when the tissue is too sticky or gelatinous to be readily handled in frozen sections.

Staining.—While the 50 year old hematoxylin-eosin and methylene blue-eosin methods are reliable and fool proof, they tend to create a "red and blue" attitude on the part of the pathologist, and they are inadequate when a discriminative diagnosis is needed. The routine use of Masson's trichrome method, using light green for the connective tissue stain, has been found to be eminently satisfactory and is strongly recommended. It makes for rapid orientation, rapid recognition of muscle or nerve tissue, and so on. Although it takes a little more time and care, it may be mastered easily and rapidly and pays for itself in the end. It cannot succeed on poor, thick sections. Special stains for mucus, pigment, fat, glycogen and like materials have been devised and should be much more frequently and generally employed. The pathologist should be at least as well versed in these matters as his technician is; otherwise he cannot intelligently direct the carrying out of special methods and is at the mercy of his technical staff. Silver impregnations are invaluable in connection with central nervous tissue and reticulum. The impregnation of the latter is particularly valuable in study of tumors of the lymphoid series.

Biopsies.—Aspiration biopsies are of distinct value, even if one must learn to make the diagnosis empirically. Centrifugated sediments from ascitic or other fluids yield valuable data for the diagnosing of metastatic tumors, particularly carcinoma.

Machinery in the Laboratory.—Although this is a machine age, machines are little used in pathologic laboratories. Mechanical tissue changers, electrical band saws and rotary saws, properly guarded, are valuable adjuncts in the laboratory and necropsy room. It is questionable whether the motorization of the microtome will become popular, as cranking by hand seems to be the method of choice and affords more delicate control.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Feb. 8, 1937

CARL W. APPELBACH, *President*

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THE TIME FACTOR IN THE IRRADIATION OF MALIGNANT TUMORS. P. J. MELNICK and ALBERT BACHEM.

This paper will be published in full in a later issue of the ARCHIVES OF PATHOLOGY.

DISCUSSION

S. ROSENTHAL: I have examined some of the sections of irradiated tumor tissues in the Coutard and Regaud collection at the Pasteur Institute, Paris. The nuclear changes are similar to those described here. The stroma of the malignant tumor seems to have some relation to its evolution. With abundant well preserved stroma, the growth of the tumor is inhibited.

E. A. PRIBRAM: Do the large forms of tumor cells represent a proliferative or a degenerative process?

P. J. MELNICK: Any changes in the stroma of these tumor tissues were not considered to be primary. The principal effect of the radiation was on the tumor cells. There are, of course, published statements emphasizing the importance of the stroma in the malignant tumor. The large cells are regarded as showing a lethal mutation. They seem to degenerate in a specific manner, the end-result being calcification.

ACTIVE IMMUNIZATION OF ALBINO RATS WITH CHEMICAL FRACTIONS FROM THE CAT TAPEWORM (TAENIA TAENIAFORMIS) AND ITS LARVAL FORM (CYSTICERCUS FASCIOLARIS [CRASSICOLLIS]). D. H. CAMPBELL.

Preliminary experiments demonstrated that rats could be immunized against infection with the larvae of the cat tapeworm by injections of whole worm material. The present study deals with the protective action of various chemical fractions of the whole worm material. Intraperitoneal injections of whole worm material (adult or larval) or chemical fractions of the whole worm were found to stimulate the rat organism to varying degrees of resistance to infection with the larvae of the cat tapeworm. Whole worm or such chemical fractions as "globulin," "nucleoprotein," substances soluble and insoluble in dilute saline solution, and "albumins" from fresh worm material produced high resistance. However, the resistance induced by the fractions was considerably less than that induced by the whole worm material. Albuminoids and polysaccharides produced resistance of a significant degree, but albumins from dried worm material were found to have little value.

The total resistance consisted of an early and a late phase, which seemed to vary independently of each other. The early resistance was measured by the difference between the total number of cysts in the controls and the total number in the immunized animals. The late resistance was measured by the number of so-called dead cysts, representing parasites which had successfully reached the liver and become encysted but were destroyed later.

Apparently, the different chemical fractions vary in their ability to induce resistance. Furthermore, some fractions induce a mechanism which either prevents larvae from reaching the liver or destroys them before they become encysted, while the protection induced by other fractions is dependent on a mechanism which destroys the larvae after their encystation.

CHEMICAL REACTION BETWEEN OLEIC ACID AND AQUEOUS SOLUTIONS OF MAGNESIUM. P. J. HARTSUCH.

A study was made of the following chemical equilibrium:

$$\underset{(Aq.)}{Mg^{++}} + 2\ \underset{(oil)}{C_{17}H_{33}COOH} \rightleftarrows \underset{(oil)}{Mg(C_{17}H_{33}COO)_2} + 2\ \underset{(Aq.)}{H^{+}}$$

To this end, 200 cc. volumes of aqueous solutions of magnesium phosphate ($MgHPO_4$) having a magnesium content of from 0.02 to 0.1 Gm. per liter and a p_H of from 5.7 to 7.9 were agitated with a known weight of pure oleic acid for from twenty-four to forty-eight hours. The p_H of the aqueous phase was redetermined, and both phases were analyzed for magnesium. The extent of the reaction was found to depend on the concentration of magnesium in the aqueous phase, the p_H and the relative amounts of the two phases. At a p_H of 5.7 or lower, almost no reaction occurred. As the p_H of the solution was increased from about 5.7, a larger and larger percentage of magnesium was transferred from the aqueous solution to form magnesium oleate dissolved in the excess of oleic acid present, with an equivalent transfer of hydrogen ions in the reverse direction from the oleic acid "oil phase" to the aqueous solution, thus lowering the p_H of the aqueous solution. In some experiments 35 per cent or more of the magnesium was lost from the aqueous solution, and the p_H fell as much as 1.63.

CLASSIFICATION OF ARTHRITIS BASED ON PATHOLOGIC OBSERVATIONS IN THE JOINTS. M. A. BERNSTEIN.

The differences in the gross appearance of atrophic, hypertrophic and proliferative arthritis were reviewed by means of lantern slide projections. The futility of internal medication in these disorders was emphasized. Surgical removal of exuberant synovial tissues was recommended.

DISCUSSION

I. PILOT: Have the synovial tissues been examined for bacteria by making cultures or stains? Many arthritides begin as infections and become degenerative processes.

A. A. GOLDSMITH: Was there ankylosis of these joints?

M. A. BERNSTEIN: I should have stated clearly that my study concerned only the noninfectious forms of arthritis. Probably many osteo-arthritides without focal infections are due to metabolic disturbance. So long as the joint surfaces are not denuded of cartilage there is no ankylosis.

Book Reviews

L'infection bacillaire et la tuberculose chez l'homme et chez les animaux. Etude biologique et expérimentale. Vaccination préventive. By A. Calmette. Fourth edition, entirely reviewed and completed by A. Boquet and L. Nègre, chefs de service in the Pasteur Institute, Paris. Price, 175 francs. Pp. 1024, with 24 plates in color. Paris: Masson & Cie, 1936.

The nine years since the appearance of the preceding edition of this book have witnessed a steady accumulation of facts in most of the fields of research on tuberculosis. In carrying out the wish of their former teacher and director, the late Albert Calmette, and bringing this book to date, the present authors have covered and recorded concisely a vast amount of recent literature. Indeed one criticism may be made in this connection, viz., that in adding the new they have failed to discard much outdated material from earlier editions with which they might dispense. Much of this literature, so carefully gathered previously, is now of historical interest only. Sooner or later, in order to keep future editions within manageable size, much of it must be omitted.

The plan of the book is essentially that originally laid out by Calmette, but in the chapters added one cannot miss a freshness of style, a quick interest in the modern and a deep devotion to the subject, that are quite the authors' own.

The sections of most interest in the new edition are those dealing with the filtrable elements of the tubercle bacillus, the dissociation and chemistry of the bacillus, spontaneous tuberculosis in laboratory animals and BCG vaccination. While the authors devote much space to recent research on the "filtrable virus" of tuberculosis and are convinced of its existence and of its ability to pass the placenta, they are cautious in according it much significance in human tuberculosis. They follow Calmette in granting that "infection after birth, within the infected family, is a factor of infinitely greater importance." In summing up the congenital aspects of tuberculosis Boquet and Nègre accord less importance than Calmette to hereditary predisposition. The attention given to familial transmission of infection, however, is not extensive; the literature cited is old, and the enormous modern statistical development of this field has not been noted.

In general, the laboratory sections of the work are the most modern. They are superior to those based on the clinic and human pathologic anatomy, reflecting the noteworthy position of the authors in the former field. Dissociation of tubercle bacilli is discussed at length, and full credit is given to Petroff for his early work in this field, in spite of difference of opinion with Petroff on the subject of BCG vaccination. Much attention is given to the chemistry of tuberculin and the proteins and lipins of the bacillus. The discussion of the first two is valuable and seems to the reviewer much better than that of the lipins. In the latter field recent work is so much superior to the old that space would be saved and a better balance struck by omitting much of the former work.

The chapter on spontaneous infection of laboratory animals is excellent. In the preceding edition Calmette wrote, "The guinea pig never, or almost never, acquires tuberculosis spontaneously." Recent investigation has shown this statement to be erroneous, and the authors have gone to considerable length to bring out the fact. In so doing they strengthen the position of BCG, casting more than reasonable doubt on certain experiments that seemed to indicate an abrupt acquisition of pathogenicity of BCG for guinea-pigs.

For laboratory investigators, clinicians and epidemiologists alike, the chapters on BCG are of the greatest interest. This section of the book has grown steadily since the first edition and now reaches 110 pages. In it will be found the latest data on methods of preparation and administration of BCG, the state of allergy induced, the extent and duration of the increased resistance to infection and advice on revaccination, all of which are of increasing interest.

This book is one of the standard works on tuberculosis, and no medical library should be without it. For the specialist on tuberculosis it is essential. The new authors have maintained its high tradition.

The Fundamentals of Electrocardiographic Interpretation. J. Bailey Carter, Clinical Instructor, Department of Medicine, Rush Medical College, The University of Chicago; Associate Staff, Cook County Hospital and Augustana Hospital, Chicago. Cloth. Price, $4.50. Pp. 326, with 250 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

This is really a reprint, with a few alterations, of articles that appeared some time ago in *The Journal of the American Medical Association*. The purpose is to aid, not the investigator, but the general practitioner in the interpretation of the electrocardiogram. Dr. Carter has drawn on his own rich experience for most of the 249 illustrations, which are fairly satisfactory, and for the case histories. In the discussion of the significance of the electrocardiogram he has made free use of the writings of others. He is to be commended for stressing the view that the electrocardiogram is not the sole basis for the diagnosis of cardiovascular conditions, that it is really an adjunct, though an important or even an essential one, to other means such as history, physical examination and roentgen examination. He is also to be commended for his wise conservatism in evaluating the many deviations from the supposedly normal that are so frequently seen in tracings. Some of these slight variations are within normal limits or are at least not significant of serious damage to the heart. This attitude of conservatism and desire to be cautious and strictly truthful are partly responsible for what is a weakness in the book. So many exceptions to rules are cited that the practitioner will often be in doubt as to what view to take concerning inverted T waves, prolonged conduction time, slight slurrings, etc. A judicious rearrangement of material, elimination of needless repetition and relatively inconsequential exceptions, with a little more dogmatism—still within the bounds of truth—would have contributed to clarity with resulting advantage to the reader. A somewhat similar fault is seen in the voluminous bibliography that—without titles of the various articles—covers about 75 pages. More care in selection, with inclusion of some important articles that are omitted and omission of many unimportant ones now included, together with a rearrangement of headings, would make the list much more useful to the practitioner, who would like to have the author select a few helpful articles rather than to be offered a bewildering long list from which he himself is to select.

This volume is written for clinicians. Pathologists may not care to read it in full. A glance at it, however, will show them how important it is for the further advance of knowledge that lesions found at autopsy should be described as accurately as possible as to location, size, apparent age, histologic nature, etc. Only in this way can there be secured such correlation of clinical features and pathologic lesions as will permit of accurate clinical diagnosis, in which electrocardiography plays such an important part.

The Use of the Developing Egg in Virus Research. By F. M. Burnet. Medical Research Council, Special Report Series, no. 220. Price, 1 shilling. Paper. Pp. 58, with 6 illustrations. London: His Majesty's Stationery Office, 1936.

This report deals with the cultivation of viruses in the chorio-allantoic membrane of the embryo chick in the developing egg. It reviews thoroughly the results obtained with the method and indicates the lines on which advances are now being made. The method was first used by Rous and Murphy in 1911 for a study of certain fowl tumors. Twenty years later Goodpasture and Woodruff showed that the viruses of fowl pox, vaccinia and herpes produced good growths on the chorio-allantoic membrane. Since then many other viruses have been added to this list. Viruses, even those not infective for fowls, may multiply freely in the membrane or in the embryo without bacterial contamination. Some viruses pro-

duce focal lesions, so that the potency of specific antiserums can be tested by counting foci. The morphologic reactions to viruses are easily followed. Study of the change in character of viruses on prolonged passage through egg membranes and embryos also is yielding interesting results. Thus it has been found that viruses may become avirulent but retain their immunizing properties. The report will be of interest and value to all who are concerned with the use of the developing egg in research on viruses.

Report of the Medical Research Council for the Year 1935-1936. Presented by the Lord President of the Council to Parliament by Command of His Majesty February 1937. Price 3 shillings net. Pp. 172. London: His Majesty's Stationery Office, 1937.

This, the twenty-second annual report, gives a detailed account of the activities of the Medical Research Council in 1935-1936. The main support for the year was a grant from Parliament of 165,000 pounds. In the first part of the report are brief reviews of the work on transmission of nerve impulses by Sir Henry Dale, the director of the National Institute for Medical Research, and of Professor Otto Loewi, of the University of Graz, for which they were awarded the Nobel Prize in Medicine for 1936; of the relations of the council to whole time clinical research; of the progress of the work under the council on influenza, nutrition, childbed fever, deafness, experimental epidemics, cotton dust "asthma," tuberculosis and pneumothorax, and of plans for developing medical research in the tropics. The rest of the report deals with the National Institute for Medical Research, the determination of biologic standards and the methods for biologic assay and measurement, clinical research units, external research schemes, industrial health, traveling fellowships, membership and representation. The report is fully indexed. Like previous reports, this report contains a great deal of interest to those concerned with medical research and its organization on a comprehensive scale as well as with its conduct and progress in special fields.

Books Received

AIDS TO PATHOLOGY. Harry Campbell, M.D., F.R.C.P., and Kenneth Campbell, O.B.E., M.B., F.R.C.S. Seventh edition. Price, $1.50. Cloth. Pp. 263, with 12 illustrations. London: Bailliere, Tindall & Cox, 1936.

REPORT OF THE HEARING COMMITTEE. V: HEARING AND SPEECH IN DEAF CHILDREN. Phyllis M. T. Kerridge. Medical Research Council, Special Report Series, no. 221. Price, 2 shillings. Pp. 137. London: His Majesty's Stationery Office, 1937.

THE FUNDAMENTALS OF ELECTROCARDIOGRAPHIC INTERPRETATION. J. Bailey Carter, Clinical Instructor, Department of Medicine, Rush Medical College, the University of Chicago; Associate Staff, Cook County Hospital, and Augustana Hospital, Chicago. Price, $4.50. Cloth. Pp. 326, with 250 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

AUTOPSY DIAGNOSIS AND TECHNIQUE. A MANUAL FOR MEDICAL STUDENTS, PRACTITIONERS, PATHOLOGISTS AND CORONER'S PHYSICIANS. Otto Saphir, M.D., chairman, Nelson Morris Institute for Medical Research; Pathologist, Michael Reese Hospital; Associate Professor of Pathology, University of Illinois Medical School, Chicago. Foreword by Ludvig Hektoen, M.D. Price, $5.00. Pp. 342, with 65 illustrations. New York: Paul B. Hoeber, Inc., 1937.

CHONDROMALACIA PATELLAE. Axel Øwre, Pathological Institute, Rikshospital, Oslo (Prof. Francis Harbitz). Paper. Pp. 157, with 21 illustrations. Oslo: Nationaltrykkeriet, 1936.

DE L'EFFET DES RAYONS X SUR LES OS LONGS EN DEVELOPPEMENT ET SUR LA FORMATION DE CAL. ÉTUDE RADIOBIOLOGIQUE ET ANATOMIQUE CHEZ LE RAT. Bjarne Dahl. Paper. Pp. 149, with 45 illustrations. Oslo: Jacob Dybwad, 1936.

Meddelelser fra Dr. F. G. Gades pathologisk-anatomiske Laboratorium i Bergen, 1936.

Arbeider fra universitetets patologisk-anatomiske Institut ved Rikshospitalet for Året 1936. Bestyrer: Prof. dr. med. Francis Harbitz. Oslo.

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PAGE